MedCrave Step into the World of Research
International Journal of Complementary & Alternative Medicine

Research Article

Open Access CrossMark

Preliminary phytochemical, physico-chemical & HPTLC analysis of aanai nerunjil kudineer

Abstract

The Siddha system is an Ancient and Indigineous system of medicine followed predominantly by the people of South India. It comprises of various modes of treatment for a number of diseases. Aanai Nerunjil kudineer is a herbal drug which is indicated for many common Genito-Urinary tract disorders in the textbook, ***Gunapadam Mooligai vaguppu*** (Siddha Materia Medica). Aanai Nerunjil (*Pedalium murex*) plants and Seeds of Kothumalli (*Coriandrum sativum)* were collected from the locality of Tuticorin, Tamilnadu and were dried under sunshade and ground into coarse powder and then mixed in a 4:1 ratio respectively.

Aqueous alcoholic Extracts of the powder was made by Hot soxhlet method**.** Alkaloids, Terpenoids, Glycosides were found to be present in preliminary phytochemical investigation. The Physico-chemical analysis revealed that the LOD at 105^0c was 8.4%, Total Ash value as 10.84%, acid insoluble ash as 0.85%, water soluble ash as 3.25%, sulphated ash as 14.56%, pH of 4% aqueous solution as 8.08, volatile oil content of 0.5%. HPTLC was performed in aqueous alcoholic extract at 254nm, 366nm & 575nm and the chromatograms were recorded. The R_f peak values were found to be significant which can further assist in identification of the individual compounds.

Keywords: siddha medicine, *pedalium murex*, *coriandrum sativum*, preliminary phytochemical, physico chemical, HPTLC Analysis

Volume 16 Issue 3 - 2023

Selvamani S, Justus Antony S, Manoharan A
Department of Pothu Maruthuvam, Govt. Siddha Medical College & Hospital, India

Correspondence: Selvamani S, Department of Pothu Maruthuvam, Govt. Government Siddha Medical College & Hospital, Palayamkottai, Tamilnadu, India-627002, Tel: 9688112541, Email smsselva112@gmail.com

Received: May 04, 2023 | **Published:** May 24, 2023

Introduction

The World Health Organization has estimated that about 80% of earth's inhabitants rely on traditional medicine for their primary health care needs that primarily involves the use of plant extracts or their active components.[1] Over 248, 000 species of higher plants have been identified and from these 12, 000 plants are known to have medicinal properties.[2]The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants[3].

Kalladaippu is a common disease of urinary tract which has the following symptoms:burning micturition, urinary obstruction, low back ache, referred pain in genital organs and in tip of penis, abnormal deposits in the urine.[4] This can be merely correlated with Urolithiasis in the modern system of medicine.Gunapadam mooligai vaguppu (Siddha Materia Medica) has mentioned a treatment named AANAI NERUNJIL KUDINEER(A combination of *Pedalium murex* Linn. Plants and *Coriandrum sativum Linn.* seeds)which is indicated for Kalladaippu.[5]

Plants can protect themselves from microbes, insects and environmental changes by producing certain chemicals or secondary metabolites which are non-nutritive, and they are called Phytochemicals.[6] The major groups of phytochemicals are phytosterols, carotenoids, aromatic acid, organic acid, flavonoids, terpenoids, saponins, alkaloids, essential oils and protease inhibitors[7]. Due to certain properties like anti-microbial, anti-inflammatory, anti-genotoxic, anti-proliferative, anti-mutagenic, anthelmintic, anti-carcinogenic, and anti-oxidant, the metabolites can establish direct or indirect protection against pathogens.[8]

The Determination of Physicochemical parameters is important to determine the adulteration and improper handling of drugs. Ash values are important to analyze the identity and purity of crude drugs especially in the powder form.

The World Health Organization is emphasising the need for standardisation and scientific evaluation of herbal drugs.[9] Nowadays, several techniques have emerged in estimating the phytochemicals qualitatively and quantitatively. HPTLC stands for High Performance Thin Layer Chromatography and is a common chromatographic technique for separating non-volatile substances. HPTLC is commonly performed on glass sheets, aluminium foil and polymers that were coated with a thin layer of absorbent materials such as silica gel, cellulosic materials and aluminium oxides.[10] The mobile phase is the solvent that is involved in the separation, whereas the stationary phase is the absorbent materials. The polarity of both phases is different. Separation by the HPTLC is highly convenient as the components are separated on the plane. Separation occurs due to polarity and the fact that some migrate less than others.[11]

Materials and Methods

The Raw Drugs were freshly collected from Tuticorin District and were purified and shade dried and ground into a coarse powder using a mixer and stored in separate airtight containers for further use. The Plants were authenticated by the Experts from Department of Gunapadam, GSMC&H, Palayamkottai.

As Described in the text, the drug has to be prepared as a concoction of AANAI NERUNJIL(*Pedalium murex* Linn. Whole plant) and Coriander Seeds(*Coriandrum sativum* Linn.) taken in 8:1 ratio and boiled with water of 10x volume to the drug until it gets reduced to1/4th of the quantity and administered orally to the patients.

Preliminary phytochemical analysis

Preparation of Extract

The Powder is Extracted with solvents namely Methanol & water by Hot Soxhlet method. The solvents are removed by distillation over water bath and the last traces of the solvent are removed by distilling under vacuum. The Extract thus obtained is used for Physico-chemical and Phyto-chemical Analysis.

1. Test for Saponins

To a few mg of extract distilled water is added and shaken well. The formation of foam indicates the presence of saponin.

2. Test for Tannin

To Substance in water is added with 5 % alcoholic ferric chloride. Dark blue colour shows presence of tannin.

3. Test for Terpenoids

To a few mg of extract in chloroform, add conc. H_2SO_4. Presence of dark brown precipitate indicates the presence of terpenoids.

4. Test for Phenol

To Substance in water is added with 5 % alcoholic ferric chloride. Dark blue or green colour shows presence of phenol.

5. Test for Steroids (Lieberman Burchard Test)

To a few mg of the extract 2 ml of chloroform is added in a dry test tube. Few drops of acetic acid is added, heated and few drops of acetic anhydride and 2 drops of concentrated sulphuric acid are added. The green colour indicates the presence of steroid.

6. Test for Quinones

To a few mg of extract, add few drops of concentrated sulphuric acid. Appearance of red colour shows the presence of quinone.

7. Test for anthraquinones

To the substance aqueous ammonia or caustic soda is added. A pink colour in the aqueous layer after shaking indicates the presence of anthraquinones.

8. Test for Glycosides

Substance is treated with anthrone and concentrated sulphuric acid. On heating over a water bath, the appearance of green colour shows the presence of glycoside.

9. Test for Carbohydrates

To the sample solution, added few drops of α-naphthol and 2-3 ml conc. H_2SO_4. The appearance of reddish violet or purple ring at the junction of two liquids indicates the presence of Carbohydrates.

10. Test for Alkaloids (Dragendorff's Test)

Few mg of extract in separate test tube was warmed with 2% Sulphuric acid for 2 minutes. And it was filtered in separate test tube and few drops of Dragendorff's reagent were added. The presence of orange red precipitates indicates the presence of alkaloids.

11. Test for Lignins

Treat the substance in alcohol or water with phloroglucinol and conc. HCl, red to pink colour shows the presence of lignins.

12. Test for Flavonoid

To the substance in alcohol add 10% NaOH or ammonia. A dark yellow colour indicates the presence of flavonoid.

13. Test for Proteins (Biuret test)

To the sample solution in a test tube, add sodium hydroxide solution and then add a few drops of very dilute (1 %) copper II sulphate solution and mix gently. Appearance of purple colour indicates the presence of protein.

14. Test for Coumarin

Substance is treated with alcoholic KOH or NaOH. Dark yellow colour shows the presence of Coumarin

Physico-chemical analysis

Loss on drying at 105°C

Test for loss on drying determines the moisture content and volatile oil present in the drug. i.e., volatile oil and water drying off from the drug.

Total Ash

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

Acid-insoluble ash

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.

Water-soluble ash

Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

Sulphated ash

Sulfated ash is one of the test to perform in the pharmaceutical industry. The test is performed to measure, when the substance is ignited at certain temperature is it volatized or not. The sulfated ash is used to determine the inorganic impurities present in the substance.

Alcohol-soluble extractive

This method determines the amount of chemical constituents extracted with Ethyl alcohol from a given amount of medicinal plant material.

Water-soluble extractive

This method determines the amount of chemical constituents extracted with water from a given amount of medicinal plant material.

pH of water extract

The pH value of an aqueous liquid may be defined as the common logarithum of the reciprocal of the hydrogen ion concentration expressed in g per litre. The pH value indicates whether the water extract of the drug is acidic, neutral or alkaline. If the pH value obtained is less than 7 the water extract is acidic, if 7 neutral and more than 7, it is alkaline.

Volatile oil

Volatile oils are generally mixtures of hydrocarbons and oxygenated compounds derived from these hydrocarbons.The odour and taste of volatile oils is mainly determined by these oxygenated constituents, which are to some extent soluble in water but more soluble in alcohol.

Citation: Selvamani S, Justus Antony S, Manoharan A. Preliminary phytochemical, physico-chemical & HPTLC analysis of aanai nerunjil kudineer. *Int J Complement Alt Med.* 2023;16(3):141–146. DOI: 10.15406/ijcam.2023.16.00644

Swelling index

The swelling index is the volume in ml taken by the swelling of 1g of plant material under specified conditions.

Foaming index

The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index.

HPTLC

The optimal separation of the constituents were achieved using the solvent system Toluene: Ethyl acetate in (5:2) ratio.The extracts were applied as different tracks of different concentrations of width 8mm each on silica gel 60 F_{254} pre-coated aluminium sheets through CAMAG micro litre syringe using Automatic TLC Sampler 4 (ATS4). After sample application the plate was introduced vertically in a CAMAG developing chamber (10cm × 10cm) pre-saturated with the mobile phase selected.

The developed chromatogram was air dried to evaporate solvents from the plate and the plate was kept in CAMAG Vizualizer and the images were captured under white light, UV light at 254nm(short length) and 366nm(long length). When exposed to short-wave UV light of 254 nm, UV-active compounds undergo fluorescence quenching and they are seen as dark spots on a bright background. On the other hand, compounds that absorb 366nm UV light are seen as bright spots on a dark background.[12]

Results & Discussion

The Preliminary Phytochemical analysis of Aanai Nerunjil Kudineer determined the presence of Terpenoids, Alkaloids and Glycosides which is represented by **Table 1**.

Table 1 Preliminary Phytochemical Analysis

Tests	Results
Saponins	-
Tannins	-
Phenols	-
Terpenoids	+
Alkaloids	+
Flavanoids	-
Steroids	-
Glycosides	+
Carbohydrates	-
Quinones	-
Proteins	-

The therapeutic values of the phytochemicals are as follows:

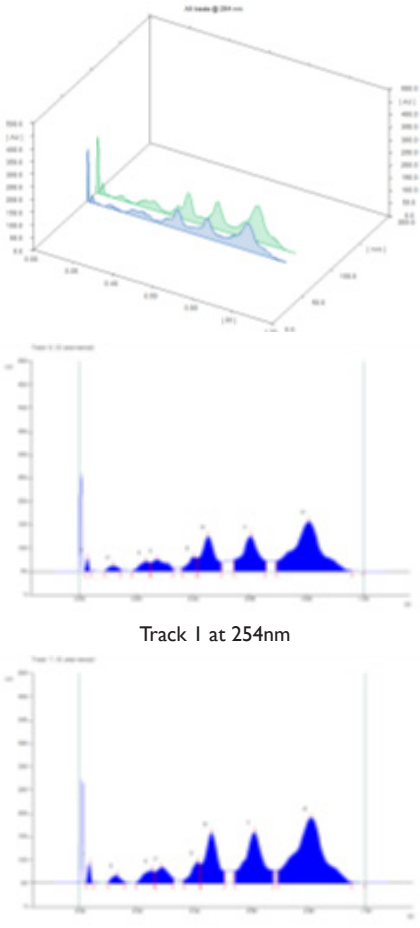
- Terpenoids are known to possess anti-tumor, anti- inflammatory,[13] anti-bacterial,[14] anti-viral, anti-malarial effects, prevent cardiovascular diseases, and hypoglycemic activities. Also, previous studies found that terpenoids are used for many applications, such as immunoregulation,[15] antioxidation,[16] antiaging and neuroprotection.
- Alkaloids are the compounds occuring naturally and known to possess anti-malarial, anti-asthmatic, hypoglycemic, anti-cancer, anti-arrhythmic, vasodilatory, cholinomimetic, anti-bacterial[17] and analgesic[18] activities.
- Glycosides are molecules obtained from plants and animals.They are known to have antifungal,[19] anticancer, antiplatelet, analgesic and anti-inflammatory activities.[20]

The Physico chemical analysis results are in Table 2. It showed that successive extract values of water as 11.72% while that of alcohol as 7.71%, loss on drying was 8.40% at 105^{0}c, total ash content was 10.84%, acid insoluble ash 0.85%, water soluble ash 3.25%, sulphated ash was 14.56%, pH of 4% aqueous solution at 8.08, volatile oil content of 0.5%.

Table 2 Physico Chemical Parameters

Sl.No	Perameters	Result
1	LOD at 105^{0}C	8.4%
2	Total Ash	10.84%
3	Acid insoluble ash	0.85%
4	Water soluble ash	3.25%
5	Sulphated ash	14.56%
6	pH of water extract	8.08
7	Volatile oil	0.5
8	Alcohol soluble extractives	7.71%
9	Water soluble extractives	11.72%
10	Swelling index	2.5
11	Foaming index	<100

The HPTLC analysis of Aanai Nerunjil Kudineer revealed the presence of various phytochemicals as illustrated in figures and tables below. The **chromatograms** (Figures 1-4) were obtained on scanning at UV 254nm, 366nm and 575nm respectively and the peak tables were generated. The R_f values, Peak area, peak height, percent areas of the unknown substances are depicted in Tables 3-8.

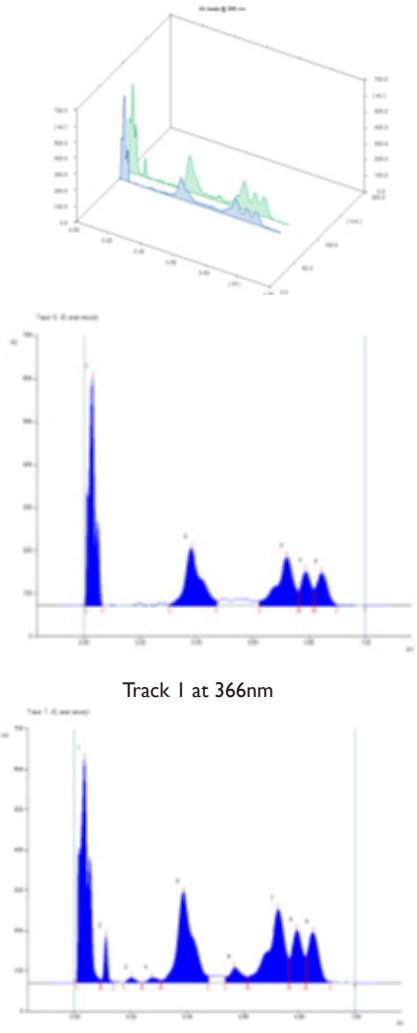


Track 1 at 254nm

Track 2 at 254nm

Figure 1 HPTLC Chromatograms scanned at 254nm.

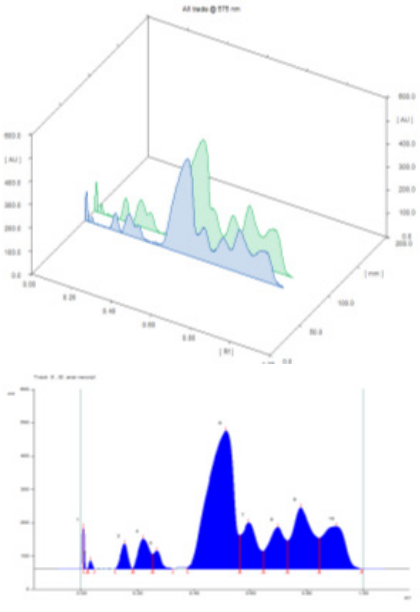
Citation: Selvamani S, Justus Antony S, Manoharan A. Preliminary phytochemical, physico-chemical & HPTLC analysis of aanai nerunjil kudineer. *Int J Complement Alt Med.* 2023;16(3):141–146. DOI: 10.15406/ijcam.2023.16.00644



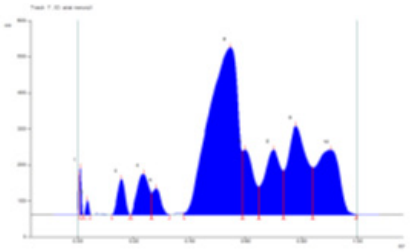
Track 1 at 366nm

Track 2 at 366nm

Figure 2 HPTLC Chromatograms scanned at 366nm.



Track 1 at 575nm



Track 2 at 575nm

Figure 3 HPTLC Chromatograms scanned at 575nm.

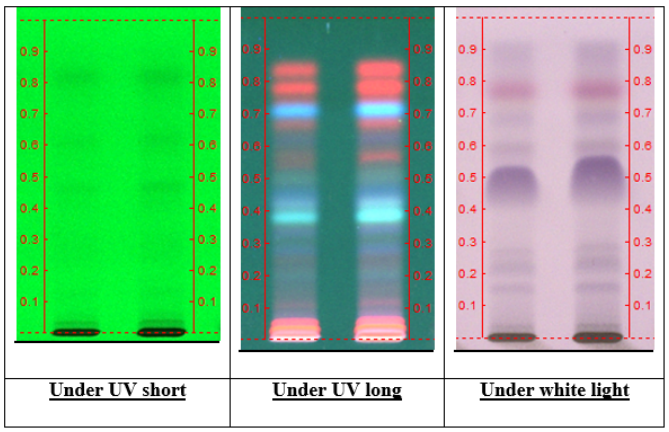


Figure 4 HPTLC Chromatograms.

The HPTLC performed in the alcoholic extracts of Aanai Nerunjil Kudineer showed the presence of various phytoconstituents in the drug in various proportions which are elucidated in the tables and figures. The chromatogram scanned at 254nm (Figure 1) wavelength showed the presence of 8&8 peaks which ranges from 0.02-0.69 & 0.03-0.69 with the major areas occupied at 47.36% and 45.91% for tracks 1 & 2 respectively.

The chromatogram at 366nm Figure 2 shows the presence of 5 and 9 peaks of ranging from 0.01-0.82 & 0.01-0.82 with the major areas occupied at 37.36% &30.83% for tracks 1 & 2 respectively.

The chromatogram at 575nm (Figure 3) of wavelength showed 10 peaks each at a range of 0.01-0.85 & 0.01-0.85 with the major areas occupied at 44.99% & 44.74% for both tracks 1&2 respectively.

The different peaks represents various phytoconstituents in the drug. The R_f values in the Tables 3-8 for the various phytoconstituents present in the drug can be used to identify unknown compounds by comparing with reference standards & from the peak areas, their concentrations can be evaluated.

The bands seen on the TLC plates representing the separation of compounds can be seen (Figure 4) visualized under white light, UV of wavelengths 254nm and 366nm.

Conclusion

As per my research work on Aanai Nerunjil Kudineer for its Preliminary Phytochemical, physicochemical & HPTLC analysis, the findings are strongly suggestive of its therapeutic value. Further researches on the drug for the identification and isolation of the compounds will be beneficial to the mankind. Therefore, this drug can be highly efficient in treating kalladaippu and prevent the recurrence of the disease.

Citation: Selvamani S, Justus Antony S, Manoharan A. Preliminary phytochemical, physico-chemical & HPTLC analysis of aanai nerunjil kudineer. *Int J Complement Alt Med.* 2023;16(3):141–146. DOI: 10.15406/ijcam.2023.16.00644

Table 3 HPTLC peak table of alcoholic extract of Aanai Nerunjil Kudineer(at 254 nm, track 1)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	1.3 AU	0.03 Rf	27.1AU	7.27 %	0.05 F Rf	0.3 AU	161.1/ AU	1.09 %
2	0.09 Rf	0.3 AU	0.12 Rf	11.3AU	3.05%	0.15Rf	3.2 AU	272.4AU	1.85 %
3	0.18 Rf	0.3 AU	0.23 Rf	21.1AU	5.68%	0.25 Rf	17.9 AU	512.0 AU	3.48 %
4	0.25 Rf	17.9AU	0.27 Rf	24.7AU	6.64%	0.33 Rf	6.0 AU	890.0 AU	6.05%
5	0.36 Rf	7.1AU	0.40 Rf	29.8AU	8.02%	0.42 Rf	29.1 AU	662.6 AU	4.50 %
6	0.42 Rf	29.2AU	0.46 Rf	75.4AU	20,27%	0.50 Rf	19.1 AU	2272.8 AU	15.44 %
7	0.55 Rf	24.5 AU	0.61 Rf	75.5AU	20,29%	0.66 Rf	23.1 AU	2975.2 AU	20.22 %
8	0.69 Rf	21.7AU	0.81 Rf	107.1 AU	28,78 %	0.96 Rf	0.5 AU	6969.4 AU	47.36 %

Table 4 HPTLC peak table of alcoholic extract of Aanai Nerunjil Kudineer(at 254 nm,track 2)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	3.3 AU	0.04 Rf	42.0 AU	8.02 %	0.05 Rf	1.5 AU	272.5 AU	1.32 %
2	0.10 Rf	0.3 AU	0.13 Rf	17.3 AU	3.29 %	0.16 Rf	2.7 AU	376.1 AU	1.82 %
3	0.19 Rf	0.5 AU	0.25 Rf	27.4 AU	5.23 %	0.26 Rf	24.0AU	730.4 AU	3.54 %
4	0.26 Rf	24.2 AU	0.29 Rf	33.9 AU	6.47 %	0.33 Rf	10.3 AU	991.3 AU	4.80 %
5	0.36 Rf	6.2 AU	0.41 Rf	45.6 AU	8.71 %	0.42 Rf	40.8AU	984.3 AU	4.77 %
6	0.43 Rf	41.1 AU	0.46 Rf	107.8 AU	20.58 %	0.51 Rf	25.4 AU	3203.8 AU	15.52 %
7	0.54 Rf	26.0 AU	0.61 Rf	109.3 AU	20.86 %	0.68 Rf	28.0 AU	4604.4 AU	22.31 %
8	0.69 Rf	28.4 AU	0.81 Rf	140.7 AU	26.85 %	0.95 Rf	2.2 AU	9476.2 AU	45.91 %

Table 5 HPTLC peak table of alcoholic extract of Aanai Nerunjil Kudineer(at 366nm, track 1)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	2.4 AU	0.03 Rf	529.4 AU	56.60%	0.07 Rf	1.0AU	8107.9AU	37.36%
2	0.31 Rf	3.4 AU	0.38 Rf	134.8 AU	14,41%	0.47 Rf	13.2AU	5150.6 AU	23,73%
3	0.63 Rf	11.3 AU	0.73 Rf	113.8 AU	12,17 ° %	0.76 Rf	34.2 AU	4382.5 AU	20.19 %
4	0.76 Rf	35.0AU	0.79 Rf	80.1 AU	8,57 %	0.82 Rf	39.6AU	1982.1 AU	9.13%
5	0.82 Rf	40.2 AU	0.85 Rf	77.2AU	8.25%	0.90 Rf	2.2 AU	2079.8AU	9.58%

Table 6 HPTLC peak table of alcoholic extract of Aanai Nerunjil Kudineer(at 366 nm,track 2)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	7.6AU	0.04 Rf	555.3 AU	39.87%	0.09 Rf	7.5 AU	11031.3 AU	30.83 %
2	0.09 Rf	7.6 AU	0.11 Rf	115.3 AU	8.27%	0.14 Rf	1.3 AU	910.0 AU	2,54 % %
3	0.17 Rf	0.1 AU	0.20 Rf	11.4 AU	0.82%	0.24 Rf	0.2 AU	227.9 AU	0,64 %
4	0.24 Rf	0.4 AU	0.28 Rf	12.0 AU	0.86%	0.31 Rf	6.0AU	286.2 AU	0.80 %
5	0.31 Rf	6.0AU	0.39 Rf	224.6 AU	16.12%	0.48 Rf	16.9 AU	8435.1 AU	23.58 %
6	0.54 Rf	14.5AU	0.57 Rf	38.2AU	2.75%	0.62 Rf	14.6 AU	1137.8 AU	3.18 %
7	0.62 Rf	14.6 AU	0.73 Rf	181.8AU	13.05%	0.76 Rf	60.1 AU	7131.6 AU	19.93 %
8	0.77 Rf	60.7AU	0.79 Rf	129.9 AU	9.33%	0.82 Rf	65.3 AU	3314.7 AU	9.26 %
9	0.82 Rf	66.3AU	0.85 Rf	124.4AU	8,93%	0.92 Rf	1.4 AU	3303.2AU	9.23 %

Table 7 HPTLC peak table of alcoholic extract of Aanai Nerunjil Kudineer(at 575 nm,track 1)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	125.1 AU	0.01 Rf	125.1 AU	9.17 %	0.02 Rf	0.0 AU	438.2 AU	0.77 %
2	0.03 Rf	1.1 AU	0.04 Rf	26.1 AU	1.91 %	0.05 Rf	0.0 AU	197.9 AU	0.35 %
3	0.12 Rf	0.1 AU	0.16 Rf	76.2 AU	5,59%	0.18 Rf	1.3 AU	1319.3 AU	2.31 %
4	0.19 Rf	2.4 AU	0.22 Rf	90.7 AU	6.65%	0.25 Rf	44.6 AU	2439.6 AU	4.27 %
5	0.25 Rf	44.7 AU	0.27 Rf	54.7 AU	4,01 ° %	0.33 Rf	0.2 AU	1077.6 AU	1.89 %
6	0.38 Rf	5.1 AU	0.51 Rf	414.0 AU	30,35 %	0.56 Rf	01.2 AU	25716.2 AU	44.99 %
7	0.56 Rf	101.3 AU	0.60 Rf	139.4 AU	10,22 %	0.65 Rf	52.9AU	5170.6 AU	9.05 %
8	0.65 Rf	53.4 AU	0.70 Rf	125.4 AU	9,19 ° %	0.73 Rf	85.5AU	4902.7 AU	8.58 %
9	0.74 Rf	85.7 AU	0.78 Rf	184.3 AU	13.51 %	0.85 Rf	92.8AU	8919.4 AU	15.60 %
10	0.85 Rf	93.0 AU	0.91 Rf	128.0 AU	9.38 %	1.00 Rf	0.2. AU	6983.9 AU	12.22 %

Citation: Selvamani S, Justus Antony S, Manoharan A. Preliminary phytochemical, physico-chemical & HPTLC analysis of aanai nerunjil kudineer. *Int J Complement Alt Med.* 2023;16(3):141–146. DOI: 10.15406/ijcam.2023.16.00644

Table 8 HPTLC peak table of alcoholic extract of Aanai Nerunjil Kudineer(at 575 nm,track 2)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	120.2 AU	0.01 Rf	129.9 AU	7.60 %	0.02 Rf	2.0 AU	623.8 AU	0.84 %
2	0.03 Rf	3.0 AU	0.04 Rf	40.3 AU	2.36%	0.05 Rf	0.0 AU	290.5 AU	0.39 %
3	0.12 Rf	0.0 AU	0.16 Rf	99.2 AU	5.80%	0.19 Rf	0.4 AU	1804.0 AU	2.43 %
4	0.19 Rf	0.8 AU	0.24 Rf	113.9 AU	6.66%	0.27 Rf	59.3 AU	3318.1AU	4,47 %
5	0.27 Rf	59.7 AU	0.28 Rf	73.0 AU	4.27%	0.33	0.1 AU	1470.9 AU	1,98%
6	0.38 Rf	5.0 AU	0.55 Rf	464.0 AU	27,16%	0.59 Rf	77.3AU	33212.5 AU	44,74 %
7	0.59 Rf	177.3 AU	0.60 Rf	181.6 AU	10,63%	0.65 Rf	77.8A	4724.2 AU	6.36%
8	0.65 Rf	78.4 AU	0.70 Rf	180.5 AU	10,56%	0.74 Rf	22.4AU	7262.2 AU	9.78%
9	0.74 Rf	122,7 AU	0.78 Rf	246.2 AU	14.41 %	0.84 Rf	31.0 AU	11457.9 AU	15.43%
10	0.85 Rf	131.0 AU	0.91 Rf	180.2 AU	10.55%	1.00 Rf	0.3 AU	10074.7 AU	13.57%

Acknowledgments

None.

Conflicts of interest

The authors have nothing to declare. They all contributed equally to the writing of the manuscript.

Funding

None.

References

1. https://www.who.int/news/item/25–03–2022–who–establishes–the–global–centre–for–traditional–medicine–in–india
2. Mukharjee PK. Quality Control of Herbal Drugs. 1st edn. Pharmaceutical Publication; 2002.
3. Kuppusamy KN, Mudaliar HPIM. Siddha maruthuvam Pothu, Dept. Of Indian Medicine & Homoeopathy, Chennai–106.
4. Kuppusamy KN, Mudaliar HPIM. Gunapadam mooligai vaguppu, Dept. Of Indian Medicine &Homoeopathy, Chennai–106.
5. Prakash B, Kumar A, Singh PP, et al. Antimicrobial and antioxidant properties of phytochemicals: Current status and future perspective. In Functional and Preservative Properties of Phytochemicals. USA: Academic Press;2020 pp. 1–45.
6. Bhattacharya S. Natural antimutagens: a review. Research Journal of Medicinal Plant. 2011;5(2):116–126.
7. Velu G, Palanichamy V, Rajan AP. Phytochemical and pharmacological importance of plant secondary metabolites in modern medicine. In Bioorganic Phase in Natural Food: An Overview. USA: Springer Science; 2018. pp. 135–156.
8. Tilburt JC, Kaptchuk TJ. Herbal medicine research and global health: an ethical analysis. Bull World Health Organ. 2008;86:594–599.
9. Factor R. Thin layer chromatograph. USA: Wiley Blackwell. 1991. p. 159–173.
10. Stahl E. Thin–layer Chromatography: A Laboratory Handbook. USA: Springer Science & Business Media; 2013.
11. https://www.merckmillipore.com.
12. Bi X, Han L, Qu T, et al. Anti– Inflammatory effects, SAR, and action mechanism of monoterpenoids from Radix Paeoniae alba on LPS–stimulated RAW 264.7 cells. Molecules. 2017;22(5):715.
13. Sebei K, Sakouhi F, Herchi W, et al. Chemical composition and antibacterial activities of seven Eucalyptus species essential oils leaves. Biol Res. 2015;48(1):7.
14. Wang TH, Hsia SM, Wu CH, et al. Evaluation of the antibacterial potential of liquid and vapor phase phenolic essential oil compounds against oral microorganisms. PLoS One. 2016;11(9):e0163147.
15. Kiuchi F, Itano Y, Uchiyama N, et al. Monoterpene hydroperoxides with trypanocidal activity from Chenopodium ambrosioides. J Nat Prod. 2002;65(4):509–512.
16. Ge JF, Li J, Hu CM, et al. Study on the immunomodulatory effects of triterpenic acid of Lycium barbarum L. Chin J Pharm. 2006;10:1194–1198.
17. Kittakoop P, Mahidol C, Ruchirawat S. Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation". Curr Top Med Chem. 2014;14(2):239–252.
18. Russo P, Frustaci A, Del Bufalo A, et al. Multitarget drugs of plants origin acting on Alzheimer's disease". Curr Med Chem. 2013;20(13):1686–1693.
19. Cushnie TP, Cushnie B, Lamb AJ. Alkaloids: An overview of their antibacterial, antibiotic–enhancing and antivirulence activities". Int J Antimicrob Agents. 2014;44(5):377–386.
20. Sinatra RS, Jahr JS, Watkins–Pitchford M. The Essence of Analgesia and Analgesics. UK: Cambridge University Press; 2010. pp. 82–90.