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ANTIOXIDANT ACTIVITY AND HPTLC ANALYSIS OF THE SIDDHA DRUG ELICEVI KUTINIR CURANAM

R. Ganesan*[1], N.Nivedha[2], T. Sujith[2], R. Shakila[1], Sampath K. S.[1] and P. Sathiyarajeswaran[1]

[1]Siddha Central Research Institute, Arumbakkam, Chennai.
[2]Valliyammal College for Women, Anna Nagar, Chennai.

***Corresponding Author: Dr. R. Ganesan**

Siddha Central Research Institute, Arumbakkam, Chennai.

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ABSTRACT

Elicevi kutinir curanam is an official siddha formulation mentioned in Balavakadam to treat intestinal worms. The present study is focused on the *In-vitro* antioxidant assay where the hydro alcoholic extract of Elicevi kutinir curanam was examined for DPPH and superoxide radical scavenging activity, Pro-oxidant effect and total antioxidant capacity. The results revealed significant antioxidant activity when compared with standard. Further the HPTLC fingerprinting profile of the extract was established which is used as a diagnostic tool for quality evaluation and standardization of the drug.

KEYWORDS: Antioxidant, DPPH (1, 1-diphenyl-2-picryl-hydrazyl), superoxide radical scavenging activity.

INTRODUCTION

Siddha Medicine (Tamil *Citta-* or *Tamil-maruttuvam*) is a system of traditional medicine in ancient Tamizhagam in South India.[1][2] Traditionally, it is taught that the siddhars laid the foundation for this system of medication. Siddhars were spiritual adepts who possessed the ashta, siddhis or the eight supernatural powers. Agastya is considered the first siddha and the guru of all siddhars; the siddha system is believed to have been handed over to him by Murugan, son of Shiva and Parvati.[3] To fight bio prospecting and unethical patents, India set up the Traditional Knowledge Digital Library in 2001 as a repository of 2,23,000 formulations of various systems medicine common in India, such as siddha, ayurveda, unani, medicine and homeopathy.[4][5] The term "antioxidant" is mainly used for two different groups of substances: industrial chemicals which are added to products to prevent oxidation, and natural chemicals found in foods and body tissue which are said to have beneficial health effects.[6][7] Elicevi kuinir curanam is a drug mentioned in Balavakadam.

The aim of the present study is to determine the antioxidant activity and HPTLC fingerprint profiles for qualitative identification of the alcoholic extract of Elicevi kutinir curanam.

MATERIALS AND METHODS

Collection of the drug

The herbal ingredients (*Merremia emarginata, Moringa olifera, Terminalia chebula, Terminalia bellirica, Phyllanthus emblica, and Emblica ribes)* for the drug were procrured from the local markets, Chennai. All the ingredients were authenticated by Dr.K.N.Sunil kumar, Research Officer (Pharmacognosy), Siddha Central Research Institute, Chennai.

Preparation of the drug

The ingredients *Merremia emarginata, Terminalia chebula, Moringa olifera, phyllanthus emblica, Terminalia bellerica, Emblica ribes* for the test drug of each 10.4g were taken and powdered and kept in a earthern pot by adding ½ portion water and allowed to boil till becomes ½ the quantity and filtered.

Extraction procedure

75g of Elicevi kutinir curanam was soaked with 500ml of hydro alcohol (1:1) mixture for 48 hours. The extract was filtered and concentrated using rotary evaporation under reduced pressure (100mbar) and reduced temperature (35c). It was transferred to a porcelain dish using minimum quantity of ethanol and diluted over water bath to free ethanol.

HPTLC methodology

Applied 10 µl of extracts on TLC plate using Camag's ATS4 applicator and developed by gradient mode the mobile phases, Methanol: 10% formic acid in Chloroform - 90:10 (developed up to 15 mm from the bottom); Methanol: 10% formic acid in Chloroform - 30:70 (developed up to 60 mm from the bottom); 10% formic acid in Ethyl acetate: toluene -60:40 (developed up to 85 mm from the bottom) using the automated multiple developer (AMD2). After development, the plate was photo documented using Camag's TLC

Visualizer under UV 254 nm and UV 366 nm. The plate was then scanned using Camag's Scanner 4 at UV 254 nm (D2 lamp, Absorption mode) finger print profiles of the extracts were documented. Then the plate was dipped in 5% sulphuric acid reagent followed by heating at 105^{o}C till development of colored spots. The plate was then photo documented in white light.

PROCEDURE

Pre-Screening method (Estimation of polyphenol content of the drug)

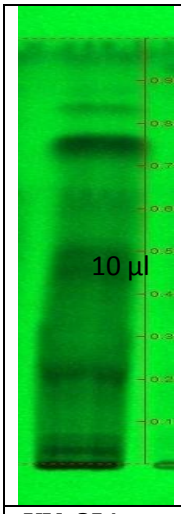
The amount of polyphenol in the drug is determined by folin-ciocalteau method. 300mg of drug was dissolved in 5ml of methanol: water: concentrated HCL (60:40:0.3). The contents were filtered through what man No.1 filter paper. After 2 minutes 100 micro liters of 50% of follins phenol reagent was added. The tubes were incubated at room temperature and the absorbance was read at 750 nm by using UV spectrophotometer. Gallic acid was used as standard. The results were expressed as milligram of Gallic acid equivalent.

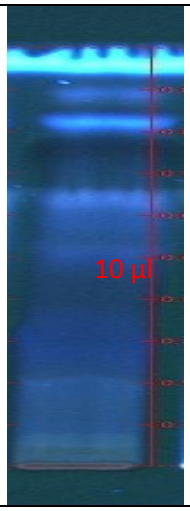
Determination of flavonoid concentrations in the drug

The content of the flavonoid in the examined drug was determined using spectrophotometric method. The drug contained 1ml of methanol solution of the drug in the concentration of 1 mg/ml and 1ml of 2% Aluminium chloride solution was dissolved in methanol. The sample were incubated for an hour at room temperature, the absorbance was determined using spectrophotometer 415nm. The same procedure for the standard solution of quercitin and the calibration line was constructed.

RESULT AND DISCUSSION

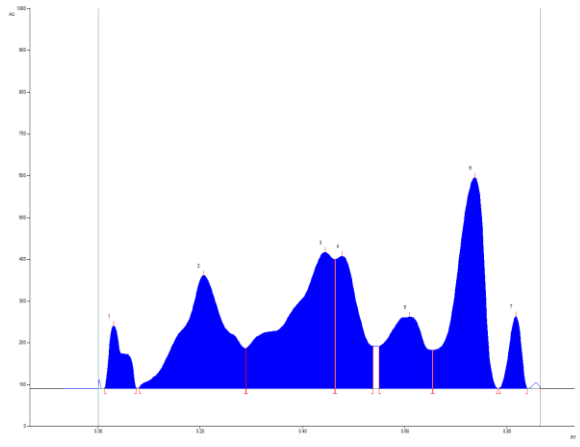
HPTLC photo documentation of elicevi kutinir curanam





UV=254 nm,	UV = 366 nm		UV = 520 nm (Derivative)		
Rf	**Colour**	**Rf**	**colour**	**Rf**	**Color**
0.04	Green	0.06	Blue	0.07	Grey
0.07	Green	0.19	Blue	0.24	Grey
0.22	Green	0.77	Dark blue	0.50	Grey
0.45	Green	0.82	Blue	0.75	Grey
0.76	Green	0.90	Blue	0.85	Grey
0.85	Green				

HPTLC Chromatogram at 254 nm **Peak Table at 254 nm**



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.3 AU	0.03 Rf	149.0 AU	7.80 %	0.07 Rf	1.2 AU	3887.0 AU	3.87 %
2	0.08 Rf	4.2 AU	0.21 Rf	270.5 AU	14.17 %	0.29 Rf	95.7 AU	20448.8 AU	20.34 %
3	0.29 Rf	96.2 AU	0.45 Rf	325.9 AU	17.07 %	0.46 Rf	08.5 AU	26061.4 AU	25.92 %
4	0.47 Rf	308.7 AU	0.48 Rf	316.1 AU	16.56 %	0.54 Rf	01.3 AU	12385.1 AU	12.32 %
5	0.55 Rf	100.8 AU	0.61 Rf	171.2 AU	8.97 %	0.65 Rf	91.6 AU	10796.8 AU	10.74 %
6	0.66 Rf	91.6 AU	0.74 Rf	504.3 AU	26.41 %	0.78 Rf	0.0 AU	23530.2 AU	23.40 %
7	0.79 Rf	0.7 AU	0.82 Rf	172.3 AU	9.03 %	0.84 Rf	0.1 AU	3431.3 AU	3.41 %

The HPTLC finger printing pattrens of the hydroalcoholic extract of Elicevi kutinir curanam was developed. The solvent system methanol 10%formic acid in chloroform (90:10), methanol, 10% formic acid in chloroform (30:70). 10% formic acid in ethyl acetate toluene (60:40). In total 7 peaks were observed and the peaks with Rf 0.21,0.45,0.74 were significant. The HPTLC finger printing can be used efficiently for identification anmd quality assessment in drug.

Pre-screening method (Estimation of polyphenol in elicevi kutinir curanam)

The Poly phenol content of the hydro alcoholic extract of Elicevi kutinir curanam were detected along with standard Gallic acid. The table 1 represents the absorbance values and percentage of inhibition for gallic acid and Elicevi Kutinir Curanam (drug) at various concentrations (10µg – 50 µg). For this method, the control value is obtained as 0.340. By using this control value, the percentage of inhibition was calculated. The percentage of inhibition standard ranges from 0.88% to 12.9% and that of the drug ranges from 0.88% to 12.94%. Thus the drug shows increase percentage of inhibition than the standard concentration.

Table 1: Absorbance and percentage of inhibition of poly phenol content in standard and drug.

S No	Concentration(µg)	Absorbance Value		% of Inhibition	
	Standard / Drug	Standard	Drug	Standard	Drug
1	10	0.337	0.337	0.88	0.88
2	20	0.330	0.330	2.9	2.94
3	30	0.305	0.305	10.2	10.29
4	40	0.302	0.296	11.1	11.17
5	50	0.296	0.277	12.9	12.94

DETERMINATION OF FLAVONOID CONCENTRATION IN ELICEVI KUTINIR CURANAM

The flavonoids content of the hydro alcoholic extract of Elicevi Kutinir Curanam were measure using quercitin as standard. The below table 2 represents the absorbance values and percentage of inhibition forquercitin and Elicevi Kutinir Curanam (drug) at various concentration (1µg – 5 µg). For this method, the control value is obtained as 0.339. By using this control value, the percentage of inhibition was calculated. The percentage of inhibition standard ranges from 20.9% to 44.2% and that of the drug ranges from 4.71% to 21.2%. Thus the drug shows the less percentage of inhibition than the standard. This may be due to less flavonoid content in the drug.

Table2: Absorbance and % of inhibition of flavonoid content in standard and drug.

S.No	Concentration(µg)	Absorbance Value		% of Inhibition	
	Standard / Drug	Standard	Drug	Standard	Drug
1	01	0.268	0.323	20.9	4.71
2	02	0.261	0.299	23	10.23
3	03	0.220	0.293	35.1	13.56
4	04	0.215	0.277	36.5	18.28
5	05	0.189	0.263	44.2	21.23

ANTIOXIDANT ASSAY

Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity of the hydro alcoholic extract of Elicevi Kutinir Curanam were measure using quercitin as standard. The below table 3

represents the absorbance values and percentage of inhibition for quercitin and Elicevi Kutinir Curanam (drug) at various concentration (5µg – 25 µg). For this method, the control value is obtained as 0.166. By using this control value, the percentage of inhibition was calculated. The percentage of inhibition standard ranges from 86.7% to 91.5 % and that of the drug ranges from 88.5% to 91.5%. Thus, the drug shows increase percentage of inhibition than the standard concentration.

Table 3: Absorbance and percentage of inhibition of DPPH content in standard and drug.

S.No	Concentration(µg)	Absorbance Value		%of Inhibition	
	Standard / Drug	Standard	Drug	Standard	Drug
1	05	0.022	0.019	86.7	88.5
2	10	0.019	0.018	88.5	88.3
3	15	0.018	0.016	89.1	90.3
4	20	0.014	0.015	91.5	90.3
5	25	0.014	0.014	91.5	91.5

Determination of the pro-oxidant effect of the drug

The pro-oxidanteffectof the hydro alcoholic extract of Elicevi Kutinir Curanam was measure using quercitin as standard. The below table 4 and graph 4 represent the absorbance values and percentage of inhibition for quercitin and Elicevi Kutinir Curanam (drug) at various concentration (5µg – 25 µg). For this method, the control value is obtained as 1.506. By using this control value, the percentage of inhibition was calculated. The percentage of inhibition standard ranges from 93.35% to 97.41% and that of the drug ranges from 5.3% to72%. Thus the drug shows the less percentage of inhibition than the standard. This may be due to less antioxidant content in the drug.

Table 4: Absorbance and percentage of inhibition of pro-oxidant effect in standard and drug.

S. No	Concentration(µg)	Absorbance Value		% of Inhibition	
	Standard / Drug	Standard	Drug	Standard	Drug
1	05	0.100	1.425	93.3	05.3
2	10	0.082	1.225	94.5	18.6
3	15	0.051	1.062	96.6	29.4
4	20	0.041	0.776	97.2	48.4
5	25	0.039	0.421	97.4	72.0

Determination of total antioxidant capacity of the drug

The total antioxidant capacity of the hydro alcoholic extract of Elicevi Kutinir Curanam was measure using ascorbic acid as standard. The below table 5 and graph 5 represent the absorbance values and percentage of inhibition for ascorbic acid and Elicevi Kutinir Curanam (drug) at various concentration (0.5µg – 2.5 µg). For this method, the control value is obtained as 0.290. By using this control value, the percentage of inhibition was calculated. The percentage of inhibition standard ranges from 1.03% to 16.5% and that of the drug ranges from 1.72% to10.24%. Thus the graph is plotted by taking x axis as concentration (µg) and y axis as absorbance. Thus the drug shows the less percentage of inhibition than the standard. This may be due to less antioxidant content in the drug.

Table5: Absorbance and percentage of inhibition of total antioxidant capacity in standard and drug.

S. No	Concentration(µg)	Absorbance Value		% of Inhibition	
	Standard / Drug	Standard	Drug	Standard	Drug
1	0.5	0.262	0.285	1.03	1.72
2	1.0	0.282	0.282	2.7	2.75
3	1.5	0.272	0.271	6.2	6.55
4	2.0	0.262	0.263	9.6	9.31
5	2.5	0.242	0.259	16.5	10.34

SCAVENGING OF SUPEROXIDE RADICAL BY ALKALINE DMSO METHOD

The superoxide radical scavenging activities of the hydro alcoholic extract of Elicevi Kutinir Curanam were measure using ascorbic acid as standard. The below table 6 and graph 6 represent the absorbance values and percentage of inhibition forascorbic acid and Elicevi Kutinir Curanam (drug) at various concentration (0.5µg – 2.5 µg). For this method, the control value is obtained as 0485. By using this control value, the percentage of inhibition was calculated. The percentage of inhibition standard ranges from52.5 to 41.2% and that of the drug ranges from 24.6% to44.3%. Thus the drug shows the less percentage of inhibition than the standard. This may be due to less antioxidant content in the drug.

Table6: Absorbance and percentage of inhibition of superoxide radical scavenging activity in standard and drug.

S. No	Concentration(µg)	Absorbance Value		% of Inhibition	
	Standard / Drug	Standard	Drug	Standard	Drug
1	0.5	0.285	0.416	41.2	24.6
2	1.0	0.273	0.403	43.5	26.9
3	1.5	0.251	0.396	48.2	28.2
4	2.0	0.243	0.354	49.89	35.8
5	2.5	0.230	0.307	52.57	44.3

SUMMARY AND CONCLUSION

The present study was performed to evaluate the HPTLC study and antioxidant assay of Elicevi kutinir curanam. HPTLC finger printing profile is a very important parameter of herbal drugs for proper identification of medicinal plants. HPTLC is a standardization method and a valuable tool for reliable identification of Elicevi kutinir curanam. The HPTLC profile of Elicevi kutinir curanam can be used as a diagnostic tool to identify and determine the quality and purity. HPTLC was performed for the hydroalcoholic extract using solvent system methanol 10% formic acid in chloroform (90:10), methanol, 10% formic acid in chloroform (30:70). 10% formic acid in ethyl acetate toluene (60:40) which gives Rf values of 0.04, 0.07, 0.22, 0.45, 0.76, 0.85 at 254nm aqnd 0.06, 0.77, 0.19, 0.82,0.90 at 366nm.

The present study demonstrates the antioxidant activity of hydro alcoholic extract of Elicevi Kutinir Curanam which is determined by the invitro assay, DPPH, Superoxide anion scavenging activity, Total antioxidant, Prooxidant activity indicated that the Elicevi kutinir curanam is a significant source of natural antioxidant. However, additional studies are necessary to develop a method for the identification and to find the compound for the antioxidant activity of the drug.

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