WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 13, 394-403. Research Article ISSN 2277– 7105

IN-VITRO ANTIOXIDANT EFFICACY AND HPTLC ASSAY OF ALCOHOLIC EXTRACT OF INCI THENURAL

M. S. Sri Harini[1], R. Ganesan*[2], S. Murugammal, R. Shakila[2], P. Sathiyarajeswaran[2]

[1]Valliyammal College for Women, Annanagar, Chennai.

[2]Siddha Central Research Institute, Arumbakkam, Chennai.

Article Received on
24 August 2017,

Revised on 14 Sep. 2017,
Accepted on 05 Oct. 2017

DOI: 10.20959/wjpr201713-9699

***Corresponding Author**
R. Ganesan
Assistant Director,
Department of Biochemistry,
Siddha Central Research Institute, Arumbakkam,
Chennai.

ABSTRACT

Inci Thenural is a combination of two herbal ingredients honey and ginger used in the treatment of kapha humour. The present study is focused on the i*n-vitro* antioxidant assay where the hydroalcoholic extract of Inci Thenural 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 can 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 system is one of the oldest systems of medicine in India. The term ‘Siddha’ means achievements and ‘Siddhars’ were saintly persons who achieved results in medicine. Eighteen Siddhars were said to have contributed towards the development of this medical system. Siddha literature is in Tamil and it is practised largely in Tamil speaking part of India and abroad.[1]

The plants have been characterized by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment.[2] These medicinal plants are considered as rich resources of ingredients which can be used in drug development and synthesis. Besides that, these plants play a critical role in the development

of human cultures around the whole world. Moreover, some plants are considered as important source of nutrition and as a result of that these plants are recommended for their therapeutic values.[3] The phenolic substances present in herb and spices have cancer chemopreventive activities, proven by both in *in vitro* as well as *in vivo* animal models.[4]

The drugs used in siddha medicine were classified on the basis of five properties: *suvai* (taste), *gunam* (character), *veeryam* (potency), *pirivu* (class) and *mahimai* (action).[5] The physiological role of antioxidants, as this definition suggests, is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals.[6] Numerous other antioxidant phytonutrients present in a wide variety of plant foods.[7] The word chromatography was derived from the greek word "chroma" which means "colour". HPTLC is used for identification or quantitation of various chemical substances from the sample taken.[8]

Therefore, it is important to assess antioxidant activity of the plants used in the herbal medicine either to elucidate the mechanism of their pharmacological action or to provide information on antioxidant activity of these herbal plants.[9]

Natural antioxidants are found in most fresh food. Natural antioxidants are molecules that are capable of slow down or preventing the oxidation process. The natural antioxidants perform the same function as a antioxidants in the body.[10,11] The aim of the present study is to determine the antioxidant activity and HPTLC fingerprint profiles for qualitative identification of the alcoholic extract of inci tenural.

MATERIALS AND METHODS

Extraction

75g of the drug (Inci Thenural) was soaked in 500ml 0f hydroalcohol (1:1) mixture for 48hours. Then, the extract was filtered and concentrated using rotary evaporator under reduced pressure (100mbar) and with reduced temperature (55 °C). It was transferred to a porcelain dish by using minimum quantity of ethanol, the drug (Inci Thenural) was dried over water bath to free ethanol.

Methodology of HPTLC

Applied 5 ul 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 upto 15 mm from the bottom); Methanol: 10% formic acid in chloroform - 30:70(developed upto 60 nm from the bottom); 10% formic acid in ethyl acetate: toluene - 70:30(developed upto 80 nm from the bottom) using the automated multiple developer (AMD2). After development, the plate was photo documented using camag's TLC visualizer under UV at 254 nm and UV at 366 nm.

Estimation of polyphenol content of the drug

The amount of polyphenol in the drug is estimated by using Folin-Ciocalteau method. 300mg of drug (Inci Thenural) was dissolved in methanol : water : concentrated-HCL in the ratio of (60:40:0.3). The contents were filtered through Whatmann No.1 filter paper. After 2 minute's 100 µl of 50% Folins phenol reagent was added to the drug extract later. The tubes were incubated at room temperature for 30 minute and the absorbance was read at 750 nm by using UV spectrophotometer. Gallic acid was used as a standard against the drug (Inci Thenural)to determine polyphenol content. Each assay was carried out in triplicate.

Determination of flavonoid content of in the drug

The concentration of flavonoid in the drug was determined by using spectrophotometric method. The drug (Inci Tenural) consist 1ml of methanol solution in the concentration range of 1 mg/ml, 1ml of 2% Aluminium chloride solution were also dissolved in methanol. The standard and test drug were incubated for an hour at room temperature after the addition of all the reagents. The absorbance was read using UV spectrophotometer at 415nm.

Antioxidant assay

Determination of DPPH radical scvanging activity

The free radical scavenging activity was determine by using 2,2-diphenyl-1-pincrylhydrazyl (DPPH) method.The reagents of DPPH solution (100mM) was prepared by dissolving 33 mg of DPPH in 100 ml of ethanol. From the stock DPPH, 10 ml of solution was taken and made upto 100 ml using ethanol (100mm) and the DPPH solution is stored in amber coloured bottle to protect from sunlight. Quercitin was used as standard. Drug (Inci Tenural) and the standard quercitin was prepared in various concentrations. various concentration of drug(0.1 ml) and 0.1 ml of DPPH solution (0.1ml ; 100mm)was incubated at room temperature for 30 minutes and the changes in absorbance in the reaction mixture was read at 517 nm.

Dertermination of pro-oxidant effect of the drug

The chemicals were prepared by dissolving 1g of potassium ferricyanide in 100 ml of distilled water (1% w/v). The phosphate buffer (0.2 M, pH-6.6) were prepared by dissolving 2.75 g of sodium dihydrogen phosphate and 5.365g of disodium hydrogen phosphate in 100 ml of distilled water. 10 g of Trichloro acetic acid (10%) was dissolved in 100 ml of distilled water and 0.1 g of Ferric chloride in 100 ml of distilled water.The drug, and the standard quercitin were used at various concentration, and these solution were mixed with 2.5 ml of phosphate buffer, potassium ferricyanide. This mixture was stored and kept at 50°C in water bath for 20 minute's. After cooling, 2.5 ml of 10% Trichloro acetic acid was added to the mixture and centrifuged at 3000 rpm for 10 minute's. Then, the supernatant of 2.5ml were mixed with equal volume of distilled water and 0.5 ml of ferric chloride solution. The absorbance was measured using UV spectrophotometer at 700 nm.

Determination of total antioxidant capacity of the drug

The reagents were prepared by using 28mm of sodium phosphate by dissolving 84 mg of sodium phosphate in 25 ml of distilled water. Ammonium molybdate(4mm) were prepared by dissolving 124 mg of ammonium molybdate in 25 ml of distilled water and 0.6 mm of sulphuric acid,100 µg of each drug was dissolved with 0.3 ml of distilled water and then the mixture was added to 3 ml of molybdate reagent containing 0.6 mm sulphuric acid, 28 mm of sodium phosphate and 4 mm of ammonium molybdate. The tubes were incubated at 95°C for 90 minute's. Then the mixture was cooled at room temperature and the absorbance was measured using UV spectrophotometer at 695 nm.

Scavenging of superoxide radical by alkaline DMSO method

The reagents were prepared with NBT by dissolving 1 mg of NBT (Nitro blue Tetra Zolium) in 1 ml of DMSO (Dimethyl Sulfoxide). 1 ml of alkaline DMSO was dissolved in 5 mm of sodium hydroxide in 1 ml of DMSO. The samples were dissolved with various concentration of 0.1 ml of NBT (1 mg / ml of DMSO) and 1 ml of alkaline DMSO (5 mm of sodium hydroxide / ml of DMSO). Then the absorbance was measured at 560 nm using UV spectrophotometer.

RESULT AND DISCUSSION

The HPTLC fingerprinting patterns of the alcoholic extract of the Inci Thenural was developed at 520 nm. The solvent system, Methanol chloroform (90:10) Methanol

chloroform (30:70) Ethyl acetate toluene (70:30) efficiently resolved the components present in the crude extract. In total six peaks were observed in the chromatogram with R_f values 0.04,0.28,0.51,0.58,0.67 (Table no: 2 and Figure no:2). The developed HPTLC chromatogram of hydro alcohol extract of the Inci Thenural may be treated as chromatographic finger printing and could be used efficiently as reference for identification, and quality assessment of this drug.

λ = 520 nm (Derivatized)	
Color	R_f value(s)
Grey	0.12
Grey	0.21
Grey	0.37

Figure 1: Peak Table at 520 nm.

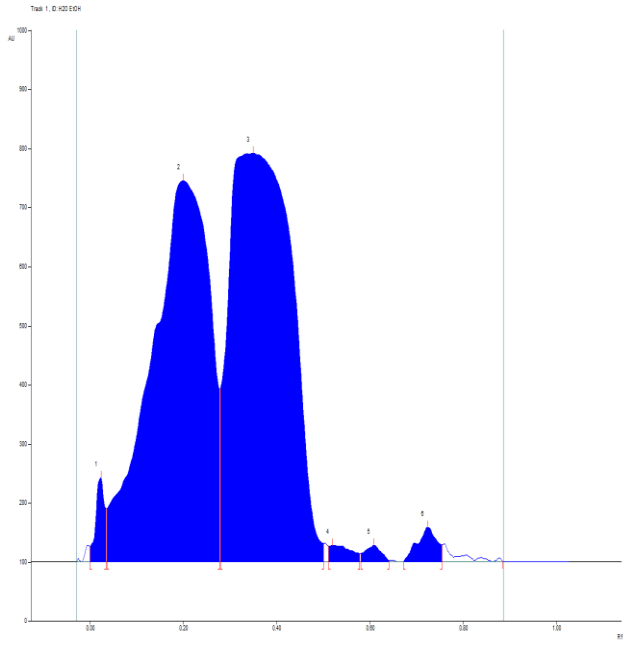

Figure 2: HPTLC Chromatogram at 520nm.

Table 1: R_f values and their percentage of HPTLC finger printing profile of Inci Thenural.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	25.6 AU	0.02 Rf	142.5 AU	8.95 %	0.04 Rf	89.7 AU	2263.2 AU	1.51 %
2	0.04 Rf	90.1 AU	0.20 Rf	644.4 AU	40.47 %	0.28 Rf	91.2 AU	66661.2 AU	44.41 %
3	0.28 Rf	292.4 AU	0.35 Rf	691.4 AU	43.42 %	0.50 Rf	31.4 AU	77310.5 AU	51.51 %
4	0.51 Rf	25.8 AU	0.52 Rf	27.7 AU	1.74 %	0.58 Rf	13.8 AU	1080.2 AU	0.72 %
5	0.58 Rf	14.2 AU	0.61 Rf	27.8 AU	1.75 %	0.64 Rf	2.7 AU	779.2 AU	0.52 %
6	0.67 Rf	0.4 AU	0.73 Rf	58.4 AU	3.66 %	0.76 Rf	28.8 AU	1999.4 AU	1.33 %

ANTIOXIDANT ASSAY

Estimation of the polyphenol content of the drug

The assay of standard gallic acid were performed and its absorbance value is found to be 0.296 and the determined percentage of inhibition is 12.9.Both the test drug and the standard gallic acid was taken with the concentration range of 10-50 ug/ml. The drug Inci Tenural of 1mg/ml was taken and the absorbance value is found to be 0.239, its percentage of inhibition is 29.70 compared against the standard gallic acid. The control value that is obtained for the drug (Inci Tenural) is 0.340. The absorbance values was read using UV spectrophotometer at 750nm.

Table 2: Estimation of the polyphenol content of the standard.

S.no	Concentration of gallic acid (mg)	Absorbance for gallic acid at 750 nm	% of inhibition
1	10	0.337	0.8
2	20	0.330	2.9
3	30	0.305	10.2
4	40	0.302	11.1
5	50	0.296	12.9

Table 3: Estimation of the polyphenol content of the drug.

S.no	Concentration of inci tenural (µg)	Absorbance for inci tenural at 750nm	% of inhibition
1	10	0.264	22.35
2	20	0.257	24.41
3	30	0.257	24.41
4	40	0.253	25.58
5	50	0.239	29.70

Determination of flavonoid concentrations of the drug

Ascorbic acid (1mg/ml) was used as a standard with its maximum absorbance value of 0.189 and the obtained percentage of inhibition was found to be 44.24. 1mg/ml of the test drug(Inci Thenural) was taken with the concentration ranges from 1-5 ug/ml and the percentage of inhibition 23.89 is calculated from the following absorbance value 0.258 against the standard ascorbic acid. The control value that is obtained for the experiment flavonoid concentration is0.339. The intensity developed was read using UV spectrophotometer at 415nm.

Table 4: Determination of flavonoid concentrations of the standard.

S.no	Concentration of ascorbic acid (µg)	Absorbance for ascorbic acid at 415nm	% of inhibition
1	1	0.268	20.9
2	2	0.261	23
3	3	0.220	35.10
4	4	0.219	35.39
5	5	0.189	44.2

Table 5: Determination of flavonoid concentrations of the drug.

S.no	Concentration of inci tenural (µg)	Absorbance for inci tenural at 415nm	% of inhibition
1	1	0.288	15.04
2	2	0.283	16.51
3	3	0.272	19.76
4	4	0.268	20.94
5	5	0.258	23.89

Determination of DPPH radical scavenging activity

In DPPH radical scavenging method, quercitin were used as a standard. The following tables represent the absorbance values and the percentage of inhibition for both standard quercitin and the test drug (Inci Tenural) at various concentration ranging from (5µg-25µg). The control value that is determined for the standard is0.166. The percentage of inhibition is calculated using the expressed control value. The percentage of inhibition is ranges from 9.63% to 18.07%. By using spectrophotometer, the absorbance values were measured at 517nm.

Table 6: Determination of DPPH radical scavenging activity of the standard.

S.no	Concentration of quercitin (µg)	Absorbance value for quercitin at 517nm	% of inhibition
1	5	0.022	86.74
2	10	0.019	88.55
3	15	0.018	89.15
4	20	0.014	91.56
5	25	0.014	91.56

Table 7: Determination of DPPH radical scavenging activity of the drug.

S.no	Concentration of inci tenural (µg)	Absorbance for inci tenural at 517nm	% of inhibition
1	10	0.150	9.63
2	20	0.145	12.65
3	30	0.141	15.06
4	40	0.138	16.86
5	50	0.136	18.07

Determination of the pro-oxidant effect of the drug

In pro-oxidant effect method, standard quercitin and the drug were taken in the range of 1mg/ml with the concentration range of(5µg-25µg). The following represented table for the standard and the drug is assinged with the measured absorbence values and the percentage of inhibition is calculated. For the standard quercitin, the control value obtained is1.506. The control value is used for the calculation of percentage of inhibition. The percentage of inhibition is ranges from 96.28% to 97.94%. By using spectrophotometer, the absorbance values were measured at 700nm.

Table 8: Determination of the pro-oxidant effect of the standard.

S.no	Concentration of quercitin (µg)	Absorbance value for quercitin at 700nm	% of inhibition
1	5	0.100	93.35
2	10	0.082	94.55
3	15	0.051	96.61
4	20	0.041	97.27
5	25	0.039	97.41

Table 9: Determination of the pro-oxidant effect of the drug.

S.no	Concentration of inci tenural (µg)	Absorbance value for inci tenural at 700nm	% of inhibition
1	5	0.056	96.28
2	10	0.051	96.61
3	15	0.051	96.61
4	20	0.035	97.67
5	25	0.031	97.94

Determination of total antioxidant capacity of the drug

Ascorbic acid was used as a standard for the total antioxiant method. The table represented with the absorbence values and the percentage of inhibition for the Ascorbic acid and the drug (Inci Tenural) at various concentration (0.5µg-2.5µg). The obtained maximum absorbance value of the drug (Inci Tenural) is found to be 0.236 with the percentage of inhibition 18.62. The control value is used to calculate the percentage of inhibition.The control value is taken from the standard ascorbic acid. The percentage of inhibition is ranges from 2.06% to 18.62%. By using UV spectrophotometer, the following absorbance values is measured at 695nm.

Table 10: Antioxidant capacity of the standard.

S.no	Concentration of ascorbic acid (µg)	Absorbance value for ascorbic acid at 695nm	% of inhibition
1	0.5	0.287	1.03
2	1.0	0.282	2.75
3	1.5	0.272	6.20
4	2.0	0.262	9.6
5	2.5	0.242	16.55

Table 11: Antioxidant capacity of the drug.

S.no	Concentration of inci tenural (µg)	Absorbance value for inci tenural at 695nm	% of inhibition
1	0.5	0.284	2.06
2	1.0	0.278	4.13
3	1.5	0.254	12.41
4	2.0	0.240	17.24
5	2.5	0.236	18.62

Scavenging of superoxide radical by alkaline DMSO method

In the superoxide radical scavening method, ascorbic acid were used as a standard. The values of absorbance and related percentage of inhibition for the drug (Inci Tenural) various with the various concentration range (0.5µg-2.5µg). For this standard, the control value is obtained as 0.552. The maximum absorbance value of the test drug is0.236 and its related percentage of inhibition is 57.24. By using this control value, the percentage of inhibition is calculated. The percentage of inhibition is ranges from 47.6% to 57.24%. By using UV spectrophotometer, the absorbance values were measured at 560nm.

Table 12: Scavenging of superoxide radical of the standard.

S.no	Concentration of ascorbic acid (µg)	Absorbance value for ascorbic acid at 560nm	% of inhibition
1	0.5	0.285	48.36
2	1.0	0.274	50.36
3	1.5	0.251	54.52
4	2.0	0.243	55.97
5	2.5	0.230	58.33

Table 13: Scavenging of superoxide radical of the drug.

S.no	Concentration of inci tenural (µg)	Absorbance value for inci tenural at 560nm	Percentage of inhibition
1	0.5	0.289	47.6
2	1.0	0.284	48.55
3	1.5	0.283	48.70
4	2.0	0.281	49.09
5	2.5	0.236	57.24

SUMMARY AND CONCLUSION

In this study the antioxidant activity of drug (Inci Tenural) showed its antioxidant index as determined by DPPH, Total antioxidant, superoxide scavenging, prooxidant methods. The drug has a rich sources of phenol having strong antioxidant capacities and could potentially replace the synthetic antioxidants in food systems and offer additional health benefits.

The drug Inci Tenural shows the good results of the antioxidant activity. Further research is needed to find the principle compounds and it's mechanism for the antioxidant activity.

REFERENCE

1. "Siddha - Origin". CCRAS, Department of AYUSH, Indian Government. Retrieved, 10 November 2011.
2. Surh YJ. Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. Food Chemical Toxicology, 2002; 40(8): 1091-1097.
3. Liu SC, Lin JT, Wang CK, Chen HY and Yang DJ. Antioxidant Properties of Various Solvent Extracts from Lychee (Litchi chinenesis Sonn.) Flowers. Food Chemistry, 2009; 114: 577-581.
4. The Siddha Formulary of India. Published by the Ministry of Health and Family Welfare, Department of Ayush, Government of India, 1992.
5. Thyagaraja Mudaliyar, Gunapadam Thaathu Jeeva vaguppu, IIIrd Edn, Indian Medical Association, 1981.
6. Halliwell B, Gutteridge JC. The definition and measurement of antioxidants in biological systems. Free Radic Biol Med, 1995; 18: 125–6.
7. Gulcin I, Alici HA, Cesur M. Determination of in- vitro antioxidant and radical scavenging activities of propofol. Pharmacology Bulletin, 2005; 53: 281-28.
8. Instrumental analysis by Skoog, Holler Nieman; 7th edition.
9. Abdul-Lateef-Molan, Abbas Mohammed Faraj, Abdulkhaliq saleh Mahdy. Antioxidant activity and phenolic content of some medicinal plants traditionally used in Northern Iraq. J of Phytopharmacology, 2012; 2(2): 224-233.
10. Smith M A, Rottkamp C A, Nunomura A, Raina A K, Perry G. Oxidative stress in Alzhiemer's disease, Biochim Biophys Acta, 2000; 1502: 139144.
11. Halvorsen BL, Holte K, Myhrstad MC, Barikino I, Hvattum E,Remberg SF, Wold AB, Haffner A. A systemic screening of total antioxidants in dietary plants. J Nutr, 2002; 132: 461-471.