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Phytochemical profiling of Tinospora cordifolia and Convolvulus pluricaulis elucidation against Acetylcholinesterase activity in Neuro-mechanism

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ABSTRACT

Applications of herbal medicines continues to escalate rapidly upon primary healthcare needs across the population worldwide. Certain natural plant products has been noted be possess therapeutic effects with negligible toxicities endowed with multitude of phytoconstituents responsible for the holistic therapeutic action.in the present study the selected plants were collected dried and extracted using the different solvents and tested for AChE inhibition by Ellman's method. Further selected extract was evaluated for the phytochemical and quantitative analysis and also antioxidant activity was estimated by DPPH and hydroxyl radical scavenging assay.

T. Cordifolia and C. Pluricaulis on successive extraction hexane yielded the11.4% and 14.7% as highest percentage of extract compared to other solvents. Among all the solvent extracts, ethanol extract of TC and CP showed impressable potency of AChE inhibition in concentration dependent manner in comparison with the other extracts respectively. The results in accordance with the respective standards shown the presence of high content of alkaloid in TPE extract (12.29±0.987) than CPE extract 3.167±0.481) whereas the flavonoid and phenolic contents were found to be more in CPE (26.36±2.030 & 68.67±2.110) extract than TCE extract (16.16±1.097 & 42.11±1.193) respectively. Even the preliminary anti-oxidant activity results in comparison with the standard show the efficiency of both the extract's potential in anti-oxidation effect with an increase in concentration.

Naturally occurring secondary metabolites, polyphenols belong to one of the most well-established classes of bioactive chemicals. Together with a wide range of phytochemicals and enzymes, they make up a substantial store of natural chemical variety. Phytochemical quantification and characterization evidences has a greater need in pharmaceutical health care system in developing nutraceutical formulations against various diseases with lesser adverse effect.

Keywords: *CPE-Convolvulus pluricaulis extract, TCE-Tinospora Cordifolia extract, AChE-Acetylcholinesterase, Phytochemicals.*

1. INTRODUCTION

Ayurvedic (Indian medical system) literature describes a class of medicinal plants known as Medhya Rasayanas. The field of herbal medicine has grown exponentially in the past several years, and due to their natural origins and low side effects, these medications are becoming more and more popular in both developed and developing nations [1]. These plants have

several uses, but one of their main purposes is to enhance memory and intelligence through Prabhava (particular action). Rasayana is a medicinal method or preparation that, when regularly practiced, will improve immunity, longevity, nutrition, health, memory, and/or intellect. Medha is the term for intellect and/or retention. Four medicinal herbs that are part of the Medhya Rasayana group can be utilised separately or in combination. These are Shankhapushpi (Convolvulus pleuricaulis Chois), Guduchi (Tinospora cordifolia (Wild) Miers), Yastimadhu (Glycirrhiza glabra Linn.), and Mandukaparni (Centella asiatica Linn.), which are specifically specified and have a wide range of applications on different systems.(2) According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency [3].

Certain organic components found in medicinal plants, such as tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids, have defined physiological effects on the human body (4,5). Living things produce these substances through their primary or more accurately secondary metabolism. Secondary metabolites are incredibly diverse substances with unknown functions in terms of chemistry and taxonomy. They are extensively employed in numerous fields, including scientific research, veterinary medicine, agriculture, and human therapy(6). Numerous phytochemicals from various chemical classes have been demonstrated to have inhibitory effects on microbes of all kinds in vitro(7). Phytomedicines have included plant-based ingredients since the beginning of time. Barks, leaves, flowers, roots, fruits, and seeds can all be used to make this(8). Understanding the chemical components of plants is important since this knowledge will be value for synthesis of complex chemical substances.(9,10,11)

In the present work, selection, authentication, extraction, ACHE activity, qualitative and quantitative phytochemical analysis and antioxidant analysis were carried out in the two selected nootropic herbs that is *Tinospora cordifolia* (Guduchi) & *Convolvulus pluricaulis* (Shankhapushpi)

2. MATERIALS & METHODS

1. Collection of plant materials and authentication:

Tinospora cordifolia and Convolvulus pluricaulis was collected in and around Mysore city outskirts. The plant was collected by consulting Dr Suma tagadur Sureshchandra, Sr. Scientific & Business advisor (Hon.), Authentic Botonicals Consultancy, Bengaluru. It was collected in the rainy season of 2019. Whole plant was collected, washed, shade dried, powdered and stored in airtight containers until further use. Plants herbarium was authenticated by Dr.Sharvani K A, and stored in Department of Botany, Yuvaraja's college, University of Mysore, with the specimen number 0282 respectively.

Fig:1 Whole plant materials were collected

Fig:2 Grounded plant materials

2. Preparation of plant extract -

Crude plant extract was prepared by Soxhlet extraction method. About 20gm of powdered plant material was uniformly packed into a thimble and extracted with 250ml of different solvents separately. Solvents used were methanol, ethanol, and acetone. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colourless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40^0 c till all the solvent got evaporated. Extracts were dried and lyophilized and stored at 4^{0}C. For experimentation, the lyophilized extracts were reconstituted using DMSO for their future use in phytochemical analysis.

Fig:3 Crude plant extract by Soxhlet extraction Method.

3. Acetylcholine esterase (AChE) activity (12) -

The extracts were used to assay the inhibition of AChE using the Ellman's method. Different concentration of extracts were added to the assay solution. Assay solution contains 0.02M DTNB, 10µL substrate ,enzyme. Incubate for 6min at room temperature with continuous gentle shake, developed yellow color was measured at 412nm.

4. Qualitative phytochemical analysis -

The extract was tested for the presence of bioactive compounds by using following standard methods [13,14,15].

Flavonoid test:

Shinoda Test: Mix Mg ribbon Pieces and HCl with extract. Pink color confirms flavonoid.

Alkaline Reagent Test: NaOH (2%) with extract. Yellow color changes to colorless on addition of diluted acid mixture confirms the presence of flavonoid.

Alkaloid test:

Dragendorff's test: Potassium bismuth iodide solution was added to extract. Orange red ppt confirms alkaloid presence.

Wagner's test: Potassium iodide and iodine mixture was diluted and added to filtrate of extract. Brown ppt confirms the presence of alkaloid.

Tannins:

5% ferric chloride was added to diluted extract filtrate. Black or blue-green coloration ppt confirms the presence of tannins.

Bromine water was added to extract. Discoloration of bromine water confirms the presence of tannins.

Glycosides:

Liebermann's Test: Acetic acid and chloroform were mixed with aqueous extract followed by addition of con H_2SO_4. Presence of green color confirms glycosides.

Steroids:

Liebermann-Burchard test: Extract was shaken with chloroform and drops of acetic anhydride was added and boiled followed by rapid cooling. Con H_2SO_4 was added. brown ring formation confirms the presence of steroids.

Saponins:

Frothing test: Shake vigorously for 5min, honeycomb froth, which was indicative of the presence of saponins.

Protein:

Hydrolysis Test: Hydrochloric acid was added followed by ninhydrin and boiled. Appearance of violet color confirms protein.

5. Quantitative phytochemical analysis :

Total phenolic content -

The amount of phenol in the aqueous extract was determined by Folin-Ciocalteu reagent method with some modifications. 2.5ml of 10% Folin-Ciocalteu reagent and 2ml of 2% solution of Na_2CO_3 were added to 1ml of plant extract. The resulting

mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 765nm. Gallic acid was used as standard (1mg/ml). All the tests were performed in triplicates. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound) [16].

Total flavonoid content -

Aluminium chloride colorimetric method was used with some modifications to determine flavonoid content. 1ml of sample plant extract was mixed with 3ml of methanol, 0.2ml of 10% aluminium chloride, 0.2ml of 1M potassium acetate and 5.6ml of distilled water and remains at room temperature for 30 minutes. The absorbance was measured at 420nm. Quercetin was used as standard (1mg/ml). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/g of extracted compound) [16].

Total alkaloid content (17)-

Preparation of reagents

Bromocresol green solution was prepared by heating 69.8 mg Bromo cresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water. Atropine standard solution was made by dissolving 1 mg of pure Atropine in 10 ml distilled water.

Extract reside was dissolved in 2N HCL & filtered. 1ml of this solution was transferred to separating funnel & washes 3 times with 10ml chloroform. The pH of this solution was adjusted to neutral by 0.1N NaOH. 5ml of BCG solution & 5ML of phosphate buffer were added to this solution. The complex was extracted with 1,2,3 & 4ml chloroform by vigorous shaking the extracts were collected in a 10ml volumetric flask & diluted with chloroform up-to the mark. The absorbance of the complex in chloroform was measured at spectrum of 470nm in UV-Spectrophotometrically against the blank prepared as above but without atropine.

6. Antioxidant assay:

a) DPPH method: The extract was used to scavenge DPPH by Kateree & Eloff(2005) described method.the extract were mixed with 4ml of a 0.006% MeoH solution of DPPH. Water/ methanol(0.2ml) in place of extracts was used as control. Absorbance at 517nnm was determined after 40min.radical scavenging activity is expressed as the inhibition percentage.(18)

b) Hydroxyl radical scavenging method: the activity of the extract was done by Kunchandy & Rao via Fenton reaction. The extract mixed with the reaction mixture containing deoxyribose in phosphate buffer(ph 7.4), ferric chloride, EDTA, ascorbic acid, hydrogen peroxide. To this 1% TCA ,2.8% TBA of equal volume were added & further incubated at 1000°C for 20min. the absorbance was measured at 532nm. Percentage inhibition was calculated by comparing the absorbance of control & test. Tocopherol was used a s reference standard.(19)

3. RESULTS & DISCUSSION -

A. Extraction of *T. Cordifolia* and *C. Pluricaulis* whole plant with range of solvents. The stored powdered plant materials were extracted using the range of solvent system with the increasing polarity. Five major solvents were used in the extraction, namely Hexane, Chloroform, Ethyl acetate, Acetone and ethanol. The obtained yield from the continuous extraction is been reported in Table-1 & Fig 4. *T. Cordifolia* and *C. Pluricaulis* on successive extraction hexane yielded the11.4% and 14.7% as highest percentage of extract compared to other solvents.

Solvents used	***T. Cordifolia* % yield (g/100g of dried whole plant material)**	***C. Pluricaulis*% yield (g/100g of dried whole plant material)**
Hexane	11.4%	14.7%
Chloroform	9%	10.2%
Ethyl acetate	6.96%	8.36%
Acetone	4.85%	5.11%
Ethanol	1.77%	2.03%

Table 1: Percentage yield of whole plant extracts.

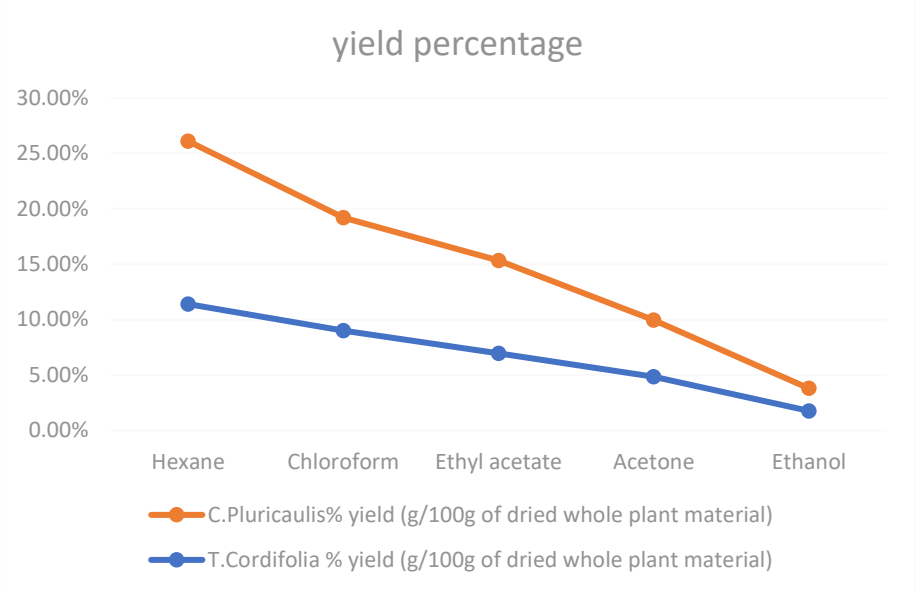
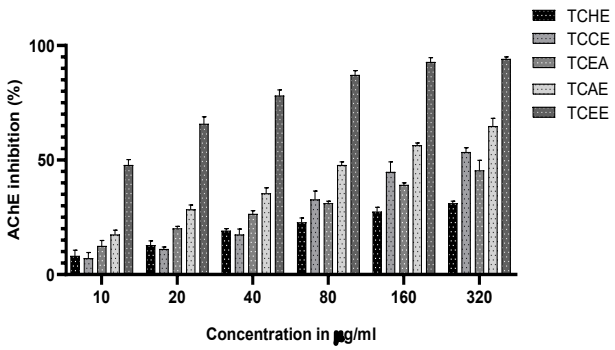


Fig 4: Comparison of extract percentage in different solvents.

B. Screening for Nootropic solvent extract -

Different solvent extracts were screened for its nootropic effects by evaluating its efficiency through AChE inhibition. AChE is the main enzyme involved in brain physiology and in cognitive functions, hence its inhibition was examined by all the solvent extracts. All the solvent extracts of TC and CP plants with range of concentrations were treated with AChE in in-vitro condition. Among all the solvent extracts, ethanol extract of TC and CP showed impressable potency of AChE inhibition in concentration dependent manner in comparison with the other extracts respectively (Figure- 5 & Figure-6)



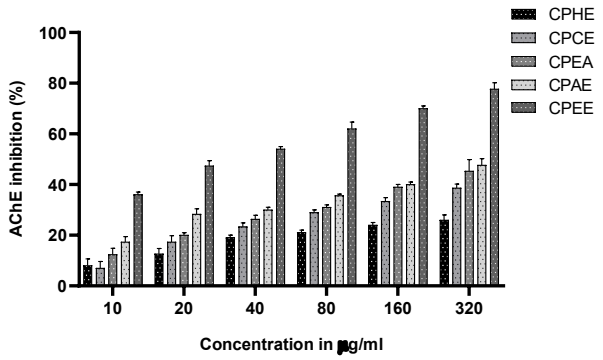


Fig:5 Effect of TC different solvent extracts on AChE activity. Data represented the mean ± SEM (n=3)

Fig: 6 Effect of CP different solvent extracts on AChE activity. Data represented the mean ± SEM (n=3)

To investigate the phytochemical properties in the targeted Nootropic herbs using analytical studies.

C. Preliminary phytochemical screening of the TCEE and CPEE:

Potent TCEE and CPEE extracts were screened for the presence of phytochemicals and are depicted in Table-2 As the extracts contains the major components contributing for the biological activity, the possible phytochemical groups were assayed with the specific phytochemical tests.

Major phytochemical	TCEE	CPEE
Alkaloid	**(P)**	**(A)**
Flavonoid	**(P)**	**(P)**
Tannin	**(P)**	**(P)**
Glycosides	**(A)**	**(A)**
Saponnins	**(A)**	**(A)**
Steroid	**(P)**	**(A)**
Protein test	**(A)**	**(A)**

Table – 2: Phytochemical screening of potent TCE and CPE extracts.(P) = presence of phytochemicals. (A) = Absence of phytochemicals.

D. Estimation of the phytochemical contents in TCEE and CPEE :

Based on the preliminary screening of the phytochemicals, the content of the phytochemicals presence from the above experiment was conducted. Accordingly the standard cure was plotted using the standard phytochemicals (Figure-7) belonging to alkaloid, phenol and flavonoid phytochemical groups.

The estimated phytochemical contents were depicted in figure- 7. The results in accordance with the respective standards shown the presence of high content of alkaloid in TPE extract (12.29±0.987) than CPE extract 3.167±0.481) whereas the flavonoid and phenolic contents were found to be more in CPE (26.36±2.030 & 68.67±2.110) extract than TCE extract (16.16±1.097 & 42.11±1.193) respectively.

Plant extract	Total phenolic content (mg GA/g dry extract)	Total Flavonoid content (mg QE/g dry extract)	Total Alkaloid content (mg AT/g dry extract)
TCE	42.11±1.193	16.16±1.097	12.29±0.987
CPE	68.67±2.110	26.36±2.030	3.167±0.481

Table 3: Phytochemical contents of the potent TCE and CPE extracts respectively with respect to the standards. Data expressed in mean ± SEM

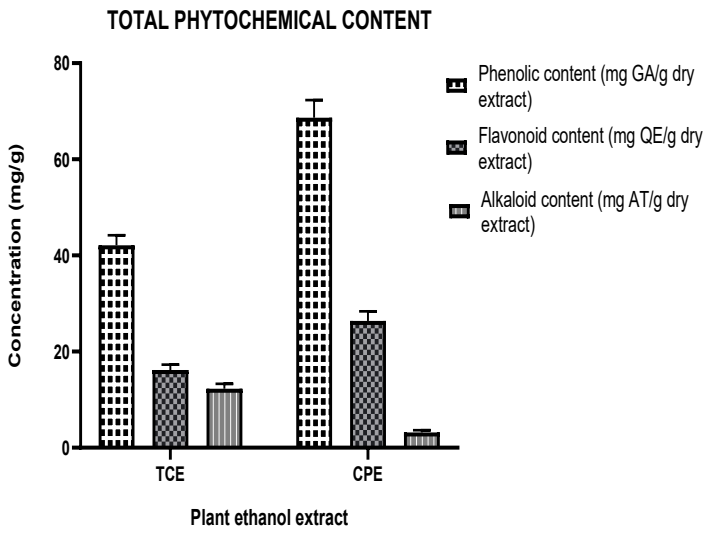


Fig 7: Estimates of total Phytochemical constituents

E. Preliminary cell free anti-oxidation bioactivity of potent TCE and CPE extracts

As the extracts contain wide range of phytochemicals, preliminary biological activity was conducted to confirm the presence of bioactive components in the extracts for further studies.

DPPH radical scavenging assay			
	Vitamin C	TCE	CPE
EC50	150.7µg/ml	137.2 µg/ml	93.99 µg/ml

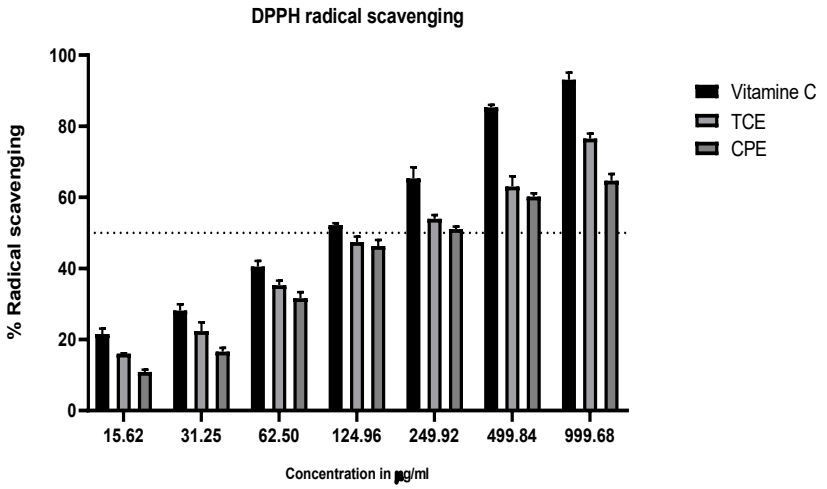


Figure-8: Efficiency of extracts in scavenging the DPPH radical. Data expressed in mean ± SEM

1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical generated in-vitro condition whereas the potent phytochemicals present in the extracts acts as a scavengers for same. The experimental results in comparison with the standard shows the efficiency of both the extracts potential in anti- oxidation effect with increase in concentration.

Hydroxyl radical scavenging assay			
	Tocopherol	TCE	CPE
EC50	107.6µg/ml	134.3 µg/ml	110.9 µg/ml

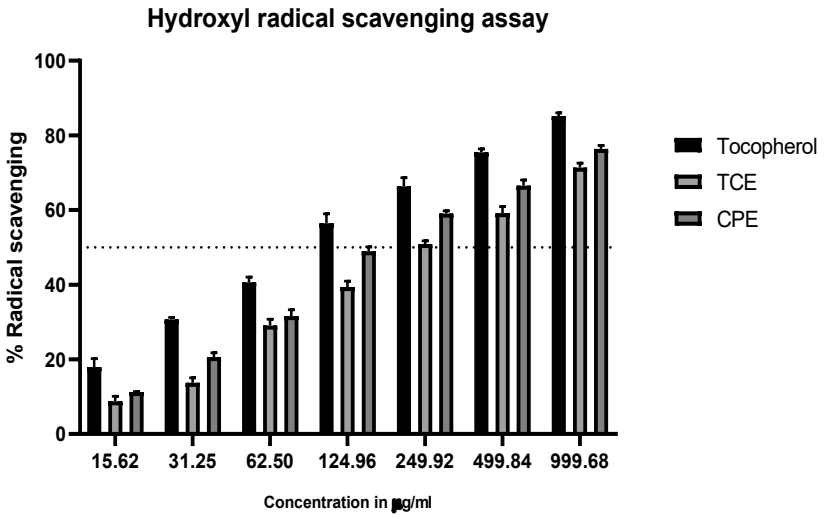


Figure-9: Efficiency of extracts in scavenging the Hydroxyl radical. Data expressed in mean ± SEM

Hydroxyl radical is the most reactive free radical, and damage the adjacent molecule. In the present assay, hydroxyl radical was generated using ascorbic acid –iron and EDTA. The generated reactive oxygen species was effectively scavenged by both the plant ethanol extract in a concentration dependent manner in comparison with the standard.

4. CONCLUSION

Ayurveda, the traditional and age-old Indian medical system, includes herbal medications as a fundamental component. One of the world's richest traditions of plant medicine is found in India. India's rural tribes are aware of around 25,000 potent plant-based remedies that are utilized in traditional medicine. Medicinal plants are important both as traditional remedies and as commodities in trade. Phenolic compounds and flavonoids are the potential substitutes for bioactive agents in pharmaceutical and medicinal sections to promote human health and prevent and cure different diseases.

Analytical study on Phytochemicals using Nootropic herbs has showed a significant presence of bioactive constituents such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. There is a marked evidences on the therapeutic actions of phytochemicals in various health morbidities. Investigation reveals a significant proportion of bioactive constituents using nootropic herbs by means of ethanol extract .

Firstly, the dried plant's samples were extracted with range of solvents from non-polar to polar region. The hexane extract of both the plants gave high yield. Further the nootropic extract was chosen by evaluating through invitro AChE inhibition. AChE is a major enzyme in the brain functioning. Typically it is noticed to help in the production of major neurotransmitter acetylcholine. It's also a common factor among many neurological disease mechanism. Hence AChE inhibition was selected as a major parameter for screening the nootropic sample. Among all the extracts, ethanol extract of T.C and C.P was considered for further analysis with 95% and 80% AChE inhibition respectively. By choosing the T.C ethanol extract (TCEE) and C.P ethanol extract (CPEE); further tested for the presence of phytochemicals contributing for AChE activity. Table-2 expressed the presence and absence of major phytochemical constituents in the extracts. Based on that the phytochemical contents was also evaluated, which showed higher content of phenolic compounds in CPEE and higher content of alkaloid in TCEE . More over a scientific literature documents has shown similar significant proportion of alkaloid in the T. Cordifolia. Additionally, cell free in-vitro assay was conducted for evaluation of the anti-oxidation efficacy of TCEE and CPEE in comparison with the standard. DPPH a stable free radical and hydroxyl radical scavenging activity was evaluated in concentration dependent manner. Results showed competence with the standard vitamin C in DPPH scavenging. Both the extracts had EC50 concentration below to the standard (150.7µg/ml) of 137.2µg/ml and 93.99 µg/ml of TCE and CPE respectively (Figure-8). While in hydroxyl radical scavenging activity, both TCE and CPE showed EC50 value of 134.3 µg/ml and 110.9 µg/ml concentration respectively. Here in comparison with the standard tocopherol (107.6µg/ml), the EC50 was found to be minute increase (Figure-9).

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