IJPSR (2020), Volume 11, Issue 4 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 06 June 2019; received in revised form, 27 August 2019; accepted, 06 February 2020; published 01 April 2020

PHYTOCHEMICAL ANALYSIS AND *IN-VITRO* ANTI-OXIDANT ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT FROM TENDER COCONUT MESOCARP

R. Elanthendral, S. Bhuvaneswari and K. Gayathri *

Department of Biochemistry, VISTAS, Chennai - 600117, Tamil Nadu, India.

Keywords:

LC-MS, DPPH, Nitric oxide radical, Phenol, Flavonoid

Correspondence to Author:
Dr. K. Gayathri

Associate Professor,
Department of Biochemistry,
VISTAS, Chennai - 600117,
Tamil Nadu, India.

E-mail: gayathri.sls@velsuniv.ac.in

ABSTRACT: Free radicals are responsible for the development of many diseases. Anti-oxidants play an important role in scavenging free radicals. Anti-oxidant activity of herbal extracts and isolated compounds are reported earlier. Pharmaceutical industries are carrying out research in the development of new potent antioxidants. Novel drug development from economically cheapest plant waste might be useful for many pharmaceutical industries. Tender coconut mesocarp (TCM) is one of the cheapest plant materials used by coir industry *etc*. Aim of the present study is to analyses various phytochemicals present in the hydroalcoholic extract of TCM and evaluate its antioxidant activity in the *in-vitro* condition. Qualitative analysis of phytoconstituents present in hydroalcoholic extract (HAE) of TCM shows the presence of carbohydrate, phenolic compounds, flavonoids and tannin. Quantitative analysis of phytoconstituents in HAE shows the remarkable amount of phenols, flavonoids, tannin and vitamin C. LCMS analysis of HAE shows the presence of (±) 13-Azaprostanoic acid with maximum ppm (13.58) and 14 other compounds with ppm > 3n were identified. The IC_{50} value of HAE to scavenge DPPH, Nitric oxide radical, Superoxide radical and hydroxyl radical has been calculated as 310.1, 289.3, 282.1, 307.6 µg/ml respectively. In conclusion, HAE is a rich source of phytoconstituents and exhibits potent free radical scavenging activity in the *in-vitro* condition.

INTRODUCTION: Free radicals are one of the important products of our own metabolism. It is an electrically charged molecules that cause cell death, penetrate through cellular membranes and react with the nucleic acids, proteins and enzymes present in the body [1]. Free radicals are obtained from metabolism or environmental sources that react continuously to the biological systems and their uncontrolled effects cause many diseases in human [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(4).1850-58

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(4).1850-58

These free radicals act as main causative agents for various disorders like arthritis, atherosclerosis, ischemia-reperfusion injury, Alzheimer's disease, Parkinson's disease, gastritis, cancer *etc.* [3] Free radicals are responsible for the pathology of many toxic effects and lipid peroxidation damages the membranes and causes cell death [4].

Reactive oxygen species (ROS) and Reactive Nitrogen Species (RNS) are the collections of a number of molecules and free radicals derived from molecular oxygen [5]. Superoxide anion, hydroxyl radicals *etc.* are formed due to metabolic reactions and also due to the exogenous sources [6]. These reactive oxygen species cause apoptosis inside cell [7]. ROS and RNS play a vital role in metabolism inside the cell that includes energy production, phagocytosis and intercellular signaling [8].

These reactive species, in turn, converted into non - reactive species by enzymatic or non-enzymatic chemical substances [9].

Reactive by-products of oxygen notably superoxide anion radical, hydrogen peroxide, and highly reactive hydroxyl radicals, are produced continuously inside the grown aerobic cells [10]. These reactive by-products cause damage to proteins, lipids and nucleotides [11]. Anti-oxidant activity of various herbs and isolated compounds are reported earlier [12]. *Cocos nucifera* belongs to the family Arecaceae is commonly referred to as "Coconut tree" and this plant is a widespread fruit tree on earth [13]. The origin of this plant was southeast Asia [14]. India is the third-largest producer of coconut and the yield was calculated as 10.56 million tonnes per year [15].

The tender coconut mesocarp is used in many coir industries [16]. The study on phytochemical analysis of an ethanolic extract from coconut oil shows the presence of phenols, tannins, flavonoids, steroids and alkaloids [17]. The ethyl acetate extract of *Cocos nucifera* fiber is a rich source of polyphenolic compounds like catechins, epicatechins, tannins and flavonoids [18]. Though various pharmacological and phytochemical evaluation of *Cocus nucifera* shell has been reported earlier, scientific evaluation of phytoconstituents and pharmacological study on tender coconut mesocarp has not been reported in previous studies.

The aim of the present study is to estimate the phytoconstituents present in the hydroalcoholic extract of tender coconut mesocarp and evaluate the antioxidant potency in the *in-vitro* condition.

MATERIALS AND METHODS:

Collection, Identification and Authentication of Tender Coconut Mesocarp: Tender coconut mesocarp was collected from Saidapet, Chennai, Tamil Nadu, India. It was identified and authenticated at the Institute of Herbal Botany and Plant Anatomy Research Centre, Chennai, Tamil Nadu, India (No: PARC/2019/3980).

Preparation of Hydroalcoholic Extract from Tender Coconut Mesocarp: Mesocarp of tender coconut mesocarp was separated and dried under shade for 7 days at room temperature. The dried plant material was finely powdered. 50.0 g of powdered tender coconut mesocarp was soaked in (70:30) ethanol: water for 72 h at room temperature. The extract was filtered and concentrated *in-vaccuo* under reduced pressure at 65 °C. The yield of Hydroalcoholic extract (HAE) of tender coconut mesocarp was calculated as 15.84 ± 0.5 g / 100 g of the plant. The dried concentrated HAE was stored in a refrigerator and used for further studies.

Qualitative Analysis of Phytoconstituents in HAE: 100.0 mg of HAE was dissolved in 100.0 mL of Ethanol: water (70:30). The presence of various phytoconstituents like carbohydrate, protein, phenol, flavonoid and tannin in HAE was qualitatively analyzed as mentioned below.

Test for Carbohydrate: 3.0 drops of HAE was used to confirm the presence of carbohydrate by subjecting to Molisch's test and Fehling's test.

Molisch's Test: 3.0 drops of HAE was treated with 2-3 drops of 1% alcoholic α-naphthol solution and 2.0 ml of concentrated sulphuric acid. The reagents were added along the sides of the test tube. The formation of a purple color shows the presence of carbohydrates.

Fehling's Test: 3.0 drops of HAE was treated with 1.0 ml of Fehling's solution (prepared by mixing equal volume of Fehling's A and B solution) and heated. The formation of orange precipitate shows the presence of reducing sugar.

Test for Proteins and Amino Acids: 3.0 drops of HAE was treated with different reagents like Ninhydrin reagent and Millon's reagent. The formation of a purple and red precipitate indicates the presence of amino acids.

Biuret Test: 3.0 drops of HAE were treated with an equal volume of solution containing 5% sodium hydroxide and 1% copper sulphate. Development of purple color indicates the presence of proteins.

Test for Phenolic Compounds: 3.0 drops of HAE was mixed with 0.5 ml of Folin phenol reagent. The tubes were allowed to stand for 5 min at room temperature and 2.0 ml of 20.0% sodium carbonate. The tubes were then kept in a boiling water bath for 5 min. The formation of blue color indicates the presence of phenolic compounds.

Test for Flavonoids: 3.0 drops of HAE was mixed with a 5.0% aqueous sodium hydroxide solution. An increase in the intensity of the yellow color indicates the presence of flavonoids.

Test for Tannins: 3.0 drops of HAE was mixed with 0.5 ml of Folin Denis reagent. The tubes were allowed to stand at room temperature for 5 min and 2.0 ml of 20.0% sodium carbonate was added. The tubes were then; kept in a boiling water bath for 5 min. The formation of blue color indicates the presence of tannins.

Quantitative Analysis of Phytoconstituents in HAE: 100.0 mg of HAE was dissolved in 100.0 ml of ethanol: water (70:30). The phytoconstituents like Phenol, Flavonoids, Tannin and vitamin C were estimated in HAE as mentioned below.

Estimation of Phenolic Compounds: The phenolic content in HAE was estimated by the method of Kavitha Chandran *et al.,* (2016) [19]. To 0.5 ml of HAE, 1.0 ml of distilled water, 2.0 ml of ammonium hydroxide and 5.0 ml of concentrated amyl alcohol were added. The sample was allowed to stand at room temperature for 30 min. The absorbance of the developed color was read at 760 nm. The results were expressed as mg of phenol/ gram of dried sample.

Estimation of Flavonoids: The total flavonoid content in the sample was estimated by the method of Chang *et al.,* (2002) [20]. 0.25 ml of HAE was diluted to 1.25 ml with distilled water. 75.0 µl of 5.0% sodium nitrite was added and after 6 min, 0.15 ml of aluminum chloride solution was added. 0.5 ml of 0.1 m NaOH was added after 5 min and made up to 2.5 ml with distilled water.

The solution was mixed well and the absorbance was read at 510 nm in comparison with standard quercetin. The results were expressed as mg of flavonoids as quercetin equivalent/gram of extract.

Estimation of Tannins: The tannin content of HAE was estimated by the method of Kavitha Chandran, (2016) [19]. 0.5 ml of HAE was made up to 1.0 ml with distilled water. To this 0.5 ml of Folin Denis reagent was added. The tubes were incubated at room temperature for 30 min. The developed blue color was read at 760 nm against reagent blank. Tannic acid was used as a standard. The values were expressed as mg of tannic acid equivalent/ gram of extract.

Estimation of Vitamin C: Vitamin C was estimated by the method of Narayanasamy *et al.,* (2011) [21]. 0.1 ml of HAE was missed with 4.0 ml of 10.0% TCA and centrifuged for 20 min at 3500 Xg. 0.5 ml of supernatant was then, mixed with 0.1 ml DTC reagent (2, 4- Dinitrophenylhydrazine (DNPH) thiourea-copper sulphate). The tubes were incubated at 37 °C for 3 h. 0.75 ml of ice-cold 65.0% H_2SO_4 was added and the tubes were allowed to stand at room temperature for an additional 30 min. A set of standards containing different concentrations of vitamin C was processed similarly along with a blank. The color developed was read at 520 nm. Values are expressed as mg of vitamin C/gram of extract.

***In-vitro* Anti-oxidant Activity of HAE:**

DPPH Radical Scavenging Activity: DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity was carried out by the method of Tuty-Anggraini (2019) [22]. To 1.0 ml of 100.0 µm DPPH solution in methanol, an equal volume of HAE in different concentrations was added and incubated in dark for 30 min. The change in coloration was observed in terms of absorbance using a spectrophotometer at 514 nm. 1.0 ml of methanol instead of the test sample was added to the control tube. Different concentration of ascorbic acid was used as a reference compound. Percentage of inhibition was calculated from the formula:

% inhibition = [(Absorbance of control - Absorbance of test)/Absorbance of control)] × 100

IC_{50} value was calculated using Graph pad prism 7.0.

Hydroxyl Radical Scavenging Activity: The hydroxyl radical scavenging activity of HAE was estimated by following the method of Mayank Gangwar *et al.,* (2014) [23]. The hydroxyl radical was generated by a fenton-type reaction. The reaction mixture contained 0.2 ml of sample in varied concentrations to which, 0.1 ml EDTA (1 mm), $FeCl_3$ (10 mm) mixture, 0.1 ml H_2O_2 (10 mm), 0.36 ml deoxyribose (10 mm), 0.33 ml phosphate buffer (50 m, pH 7.4) and 0.1 ml of ascorbic acid (1 mm) was added in sequence. The mixture was incubated at 37 °C for 1 h. To this mixture was added 1.0 ml

each of Trichloroactic acid (10%) and Thiobarbituric acid (0.67%) and kept in boiling water bath for 20 min. The color developed was read at 532 nm. The control tube contains phosphate buffer, instead of a sample. A different concentration of ascorbic acid was used as a reference compound. The percentage of inhibition and IC_{50} value were calculated as mentioned above.

Superoxide Radical Scavenging Activity: The superoxide radical scavenging activity of HAE was analysed by following the method of Vadivukkarasi *et al.,* (2014) [24] with slight modifications. Superoxide radicals are generated in phenazinemetho sulphate (PMS) - (Nicotinamide adenine dinucletide reduced (NADH) systems by oxidation of NADH and assayed by the reduction of Nitro Blue Tetrazolium (NBT). 200.0 µl of HAE in different concentrations were taken in a series of a test tube. Superoxide radicals were generated by 1.0 ml of Tris-HCl buffer (16.0 mm, pH-8.0), 1.0 ml of Nitrobluetetrazolium (50.0 µm), 1.0 ml NADH (78.0 µm) solution and 1.0 ml of Phenazine methosulphate (10 µm). The reaction mixture was incubated at 25 °C for 5 min and the absorbance was read at 560 nm against a blank. A control tube containing the Tris-HCl buffer was also processed in the same way without the test sample. Different concentration of ascorbic acid was used as a reference compound. The percentage of inhibition and IC_{50} value were calculated as mentioned above.

Nitric Oxide Radical Scavenging Activity: The nitric oxide radical scavenging activity of HAE was determined using the method of Sourav Mandal e*t al.,* (2011) [25]. At pH 7.4 nitric oxide generated from aqueous sodium nitroprusside (SNP) solution interacts with oxygen to produce nitrite ions, which may be quantified by the Griess reaction. The reaction mixture contained 10 mm SNP, phosphate-buffered saline (pH 7.4) and various concentrations of HAE to a final volume of 3 ml. After incubation for 150 min at 25 °C, 1 ml sulfanilamide (0.33% in 20.0% glacial acetic acid) was added to 0.5 ml of incubated solution and allowed to stand for 5 min.

Then 1.0 ml of napthyl ethylenediamine dihydrochloride (NED) (0.1% w/v) was added and the mixture was incubated for 30 min at 25 °C. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with NED was measured spectrophotometrically at 540 nm against a blank. Different concentration of ascorbic acid was used as a reference compound. Percentage of inhibition and IC_{50} value were calculated as mentioned above.

Statistical Analysis: Values are Mean ± SD of triplicate. Graph pad software version 7.0 was used to calculate IC_{50} value. A significant difference was observed at $p<0.01$ and calculated by using Student's't' test.

RESULTS AND DISCUSSION: Pharmaceutical industries are involved in the research and development of the novel drug. Plants are being used by pharmaceutical industries for the development of new drugs. A compound isolated from plant extracts is reported to exhibit various pharmacological activity. Traditional medical practitioners are using many infusions as healing agents for many ailments. Most of those products are scientifically evaluated. Physiochemical analysis of tender coconut mesocarp has been done to check the purity of collected material in **Table 1**.

Since mesocarp of tender coconut has been used by coir industry, an attempt has been made to estimate the amount of edible fiber present in tender coconut mesocarp and it's was determined as 7.73 g/100g. This crude fiber content of TCM has not been determined and reported in earlier studies. The crude fiber content of many plants has been reported earlier [26]. Fiber present in *Malvia sylvestris* leaves and stem exhibits laxative property and increases the fecal bulk by 105% [27]. Though TCM contains some amount of fiber, the plant material has not been eaten by people. The fibre of TCM should be isolated and its pharmacological laxative activity should be evaluated. Loss on drying, ethanol-soluble extractive value and water-soluble extractive value of TCM was found to be high in **Table 1**. The plant might be useful for the pharmaceutical industry as a source of the drug.

TABLE 1: PHYSIOCHEMICAL ANALYSIS OF TENDER COCONUT MESOCARP

S. no.	Parameters	Quantity (g/100 gm of plant material)
1	Crude fibre content	7.73 ± 0.20
2	Loss on drying	94.3 ± 1.04
3	Ethanol soluble extractive value	12.7 ± 0.41
4	Water soluble extractive value	31.1 ± 1.58

TABLE 2: QUALITATIVE ANALYSIS OF PHYTO-CONSTITUENTS IN HAE OF TENDER COCONUT MESOCARP

S. no.	Phytoconstituents	Presence/Absence
1	Carbohydrate	Present
2	Amino acid/Protein	Absent
3	Phenolic compounds	Present
4	Flavonoid	Present
5	Tannin	Present

Table 2 depicts the presence of carbohydrate, phenolic compounds, flavonoids and tannins in HAE of TCM, whereas, amino acids and proteins were found to be absent. Carbohydrate is a macromolecule and energy-giving source. The type of sugar present and the energy obtained from TCM upon digestion should be evaluated. The presence of carbohydrates in HAE of TCM might be due to the presence of cardiac glycosides.

Treatment of congestive heart failure and cardiac arrhythmia with cardiac glycoside has been reported earlier and these effects might be due to increasing the force of muscle contraction with reducing heart rate [28]. The type of cardiac glycosides present in HAE of TCM should be evaluated further. All enzymes are proteins in nature.

Protein and amino acids were observed to be absent in HAE of TCM **Table 2** and it might not be a source for any type of enzymes and amino acid-derived hormones. Phenolic compounds, flavonoids and tannins were observed to be present in HAE of TCM **Table 2** and their quantity was calculated as 3.45, 0.97 and 1.82 mg/g **Table 2**. In general, many types of phenolic compounds are produced by the plant. These compounds might be useful for the prevention of some chronic or degenerative diseases.

The pharmacological activity of phenolic compounds includes antioxidant and radical scavenging activity, hepatoprotective effect, xanthine oxidase inhibition, semicarbazide-sensitive amine oxidase inhibition, angiotensin I converting enzyme inhibition, anti-hyperuricemic effect and anti-inflammatory activity [29]. Hydroxy benzoic acid and β- D- Salicinare some of the phenolic compounds reported to exhibits its effects on inflammation, cell proliferation and apoptosis activity in various types of cell lines [30]. Most of the phenolic compounds are present in the form of flavonoids or tannins in plants. Flavonoid content of HAE of TCM has been calculated in **Table 3**. In general, flavonoids were reported to exhibit numerous health benefits.

Some flavonoids are shown to have antioxidant activity, free radical scavenging capacity, coronary heart disease prevention, hepato-protective, anti-inflammatory and anticancer activities, while some flavonoids exhibit potential antiviral activities and in plants, flavonoids act as a growth regulator [31]. Studies must be carried out to analyze the type of flavonoid present in HAE of TCM and its pharmacological activities should be evaluated. In addition to flavonoids, a major amount of tannins are present in HAE of TCM.

Generally, water-soluble polyphenol tannins are distributed in many plants. In experimental animals, tannin decreases feed intake, growth rate, feed efficiency, net metabolizable energy and protein digestibility. Therefore, tannin-rich food is less in nutritional value. However, tannin was reported to exhibit anti-carcinogenic, anti-mutagenic, anti-oxidant, anti-microbial property and protects cellular oxidative damage and including lipid peroxidation.

Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease serum lipid level, produce liver necrosis, and modulate immune responses [32]. 0.9 mg of vitamin C was determined to be present in 1.0 g of HAE from TCM.

This vitamin C might be helpful in exhibiting various pharmacological activities such as antioxidant. But the anti-oxidant effect of vitamin C may depend on the number of factors such as the redox state of the body, the dose used and also on the tissue metabolism [33]. Vitamin C content of HAE might be responsible for the antioxidant activity of tender coconut water [34].

TABLE 3: QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS IN HAE OF TENDER COCONUT MESOCARP

S. no.	Phytoconstituents	Amount (mg/g of extract)
1	Phenolic compounds	3.45 ± 0.49
2	Flavonoids	0.97 ± 0.007
3	Tannins	1.82 ± 0.106
4	Vitamin C	0.9 ± 0.08

TABLE 4: DPPH RADICAL SCAVENGING EFFECT OF HAE OF TENDER COCONUT MESOCARP

S. no.	Vitamin C		HAE	
	Concentration (µg)	Inhibition	Concentration (µg)	Concentration (µg)
1	2	20.24 ± 0.04	100	8.19 ± 0.08
2	4	25.95 ± 0.12	200	25.00 ± 0.11
3	6	48.76 ± 0.04	300	40.95 ± 12.08
4	8	75.29 ± 0.06	400	65.67 ± 0.02
5	10	87.05 ± 0.02	500	91.52 ± 0.07
	IC_{50}	5.56 *	IC_{50}	310.1

Values are Mean ± SD of triplicate. * - Significant difference ($p<0.05$) is calculated using Student's 't' test

TABLE 5: NITRIC OXIDE RADICAL SCAVENGING EFFECT OF HAE OF TENDER COCONUT MESOCARP

S. no.	Vitamin C		HAE	
	Concentration (µg)	Inhibition	Concentration (µg)	Inhibition
1	100	9.10±0.10	100	12.80±0.08
2	200	16.90±0.04	200	31.19±0.12
3	300	40.90±0.05	300	46.09±0.14
4	400	68.24±0.03	400	65.66±0.02
5	500	89.38±0.08	500	86.19±0.06
	IC_{50}	320.4	IC_{50}	289.3

Values are Mean ± SD of triplicate.

TABLE 6: SUPEROXIDE RADICAL SCAVENGING EFFECT OF HAE OF TENDER COCONUT MESOCARP

S. no.	Vitamin C		HAE	
	Concentration (µg)	Inhibition	Concentration (µg)	Inhibition
1	10	11.69±0.07	100	13.59±0.01
2	20	30.32±0.11	200	34.28±0.01
3	30	45.43±0.13	300	50.47±0.17
4	40	65.16±0.02	400	63.49±0.13
5	50	85.87±0.07	500	79.40±0.16
	IC_{50}	29.4 *	IC_{50}	282.1

Values are Mean ± SD of triplicate. * - Significant difference ($p<0.05$) is calculated using Student''s 't' test

TABLE 7: HYDROXYL RADICAL SCAVENGING EFFECT OF HAE OF TENDER COCONUT MESOCARP

S. no.	Vitamin C		HAE	
	Concentration (µg)	Inhibition	Concentration (µg)	Inhibition
1	100	24.81±0.04	100	8.52±0.04
2	200	27.38±0.13	200	19.95±0.04
3	300	53.90±0.07	300	47.38±0.08
4	400	74.71±0.10	400	69.14±0.01
5	500	88.05±0.04	500	82.90±0.22
	IC_{50}	259.9	IC_{50}	307.6

Values are Mean ± SD of triplicate.

Metabolic reactions in the cells produce various Reactive Oxygen Species (ROS) either as free radicals or non-radicals. Superoxide anion, hydroxyl radical, and hydrogen peroxide are important ROS generated endogenously. These ROS are scavenged by means of anti-oxidant. Plant extracts are rich sources of numerous phytoconstituents with potent anti-oxidants.

The anti-oxidant ability of each plant varies depending on the presence of phytoconstituents. In the present research, the anti-oxidant activity of HAE of TCM has been evaluated by the *in-vitro* study. **Table 4**, **5**, **6**, **7** shows the free radical scavenging activity of HAE from TCM and it was compared with the standard antioxidant vitamin C. DPPH and Superoxide radical has been scavenged by vitamin C significantly ($p<0.01$, **Table 5**, **7**) better than that of HAE of TCM. The IC_{50} value of nitric oxide and hydroxyl radical scavenging ability of vitamin C and HAE reveals that significant difference has not been observed in **Table 5**, **7**.

The free radical scavenging activity of HAE might be due to the presence of the phenolic compound, flavonoids and tannins in **Table 2**, **3**. The antioxidant ability of phenolic compounds is due to reduction by hydrogen-bond. The hydrogen-bonded

molecules aromatic - OH---S are virtually unreactive towards ROO* radicals [35]. Flavonoids are phenolic substances isolated from a wide range of plants. They serve in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, and feeding repellents. Anti-oxidant activity of flavonoids is due to their ability to reduce free radical formation. The capacity of flavonoids to acts as antioxidants in both *in-vitro* and *in-vivo* is reported earlier. Most ingested flavonoids are extensively degraded to various phenolic acids, some of which still possess a radical-scavenging ability [36]. Tannins serve both as primary and secondary antioxidants. Tannins have the ability to chelate metal ions such as Fe^{2+} and interfere with one of the reaction steps in the Fenton reaction and thereby retard oxidation [37].

DPPH is one of the few stable and commercially available organic nitrogen radicals. The DPPH radical scavenging assay is a simple and sensitive assay, well known for evaluating natural product antioxidant studies. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers [38]. The antioxidant effect is proportional to the disappearance of DPPH• in test samples. DPPH shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow, followed by the formation of DPPH upon absorption of hydrogen from an anti-oxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm [39]. DPPH has been scavenged by HAE with 310.1 µg as IC_{50}. This might be due to the presence of vitamin C in HAE. The vitamin C content of HAE has been calculated as 0.9 mg/g. DPPH radical scavenging activity of vitamin C has been reported earlier. Nariya *et al.*, (2013) [40] have calculated IC_{50} value of vitamin C in DPPH radical scavenging assay as 6.1 µg/ml respectively.

Superoxide anion is formed by the addition of 1 electron to the molecular oxygen by nicotine adenine dinucleotide phosphate oxidase or xanthine oxidase or by the mitochondrial electron transport system. Mitochondria are the major site for the production of superoxide anion. 1-3 of electron leaks from the electron transport chain and produces superoxide. This free radical can damage polysaccharide, lipids, proteins, nucleic acids *etc*.

Harmful superoxide radical has been scavenged by HAE with 282.1 µg as IC_{50}. This result depicts that HAE can protect biomolecules and another cellular organelle membrane from damage.

Nitric oxide is a free radical generated by endothelial cells, macrophages, neurons, and involved in the regulation of various physiological processes. Whereas excess NO causes DNA fragmentation, neuronal cell death and cell damage [41]. Nitric oxide has been scavenged by HAE at 289.3 µg as IC_{50}. This result depicts that HAE may protect DNA damage caused by Nitric oxide.

Hydroxyl radicals generated in the human body may play an important role in tissue injury at sites of inflammation in oxidative stress-originated diseases [42]. Anti-oxidant can protect the tissue from damage caused by hydroxyl radicals.

Hydroxyl radical has been scavenged by HAE at 289.3 µg as IC_{50}. The free radical scavenging and antioxidant activity of HAE might be due to the presence of various phytoconstituents like phenol, tannin, flavonoid, *etc.* **Table 2**, **3**.

CONCLUSION: In conclusion, HAE is a rich source of phytoconstituents with potent free radical scavenging activity in the *in-vitro* condition. Hence, further studies on other pharmacological activities of HAE like anti-diabetic, anti-cancer and cardioprotective should be evaluated both in *in-vivo* and *in-vitro* studies.

ACKNOWLEDGEMENT: The authors are grateful to Dr. Ishari Ganesh, Chairman, VISTAS, and his able administrative colleagues for allowing us to complete this work. We also thank other faculty of the Department of Biochemistry, VISTAS, for their valuable suggestions and assistance to complete this work successfully.

CONFLICTS OF INTEREST: The author declared no conflict of interest.

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How to cite this article:
Elanthendral R, Bhuvaneswari S and Gayathri K: Phytochemical analysis and *in-vitro* anti-oxidant activity of hydroalcoholic extract from tender coconut mesocarp. Int J Pharm Sci & Res 2020; 11(4): 1850-58. doi: 10.13040/IJPSR.0975-8232.11(4).1850-58.