Isolation of Phytochemical from *Coccus nucifera* and Evaluation of Antioxidant Potency

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Abstract

The botanical name of the coconut is *Coccus nucifera*, also called the 'Tree of Life' because each part of the coconut plant is essential to humans. Many researchers have studied coconut water, leaves, oil, flowers, and cotyledon and proved that coconut exhibits a wide range of bioactivities viz antimicrobial, antioxidant, antitumor, anti-inflammatory, antifungal, and anti-bacterial activity. However, there is less or no study on the coir or mesocarp of coconut. The objectives of the present research work were to identify the phytochemical constituents and to review the pharmacological activities of fresh coconut coir which would be helpful in the diagnosis and treatment of many diseases. In this investigation, fresh coconut coir was used to extract phytochemicals using nonpolar to polar solvents. These extracts were used for further studies such as antioxidant study, determination of total phenolic content and total flavonoid content present in the crude extract. The fresh coir shows good antioxidant activity by using 2,2-diphenyl – 1 – picryl hydrazyl (DPPH) and hydrogen peroxide as a reagent. It also shows good amounts of total phenolic content and good flavonoid content.

Keywords: Mesocarp, Phytochemicals, Antioxidant, Total Phenolic Content (TPC), Total Flavonoid Content (TFC).

Introduction

According to the World Health Organisation, about 70-80% of the world's population uses ethno medicinal plants for various purposes. The pharmacologically active compounds present in plants also play an important role in herbal medicines. Medicinal plants or herbal medicines are concerned; it has fewer side effects as compared to allopathic pharmaceutical preparations. *Coccus nucifera* commonly

known as *nariyal* belongs to the Arecaceae family. It has bioactive ingredients including phenols, carbohydrates, flavonoids, proteins lipids etc. [1]

Coccus nucifera is commonly known as coconut and is native to tropical Eastern regions and subtropical countries. It is an important member of the family Arecaceae having the order Arecales with the genus *Coccus* and species *nucifera*.

The palm is sandy, shoreline, eternal having about 100 years of life with an average height of 20–30 m. The fibrous trunk with ring-like projections indicates its growth and age. It has a fibrous root system. The adult coconut palm has 12 – 14 or sometimes 18 clusters of flowers under favourable environmental conditions. The palm is monoecious, having male and female reproductive organs on the same plant. The leaves are pinnate, feather having petioles and leaflets. Botanically coconut fruit or nariyal is a drupe, not a nut. It is fibrous consisting of pericarp and seed. The pericarp has three layers viz. outer thick fibrous Exocarp, Mesocarp/husk/coir which turns brown and dry at maturity and flawless. Coconut husks are tissues surrounding to seed. It is odourless, lightweight, thick, strong, and resistant to any injury or scratch. Husk fibres are mostly made up of cellulose, hemicelluloses, lignin and extractives [1, 2]. And a hard-shell inner layer, Endocarp. The seed comprises the seed coat, embryo, and endosperm. Endosperm is also known as kernel containing white flesh solid endosperm and liquid endosperm that is coconut water [1, 3, 4].

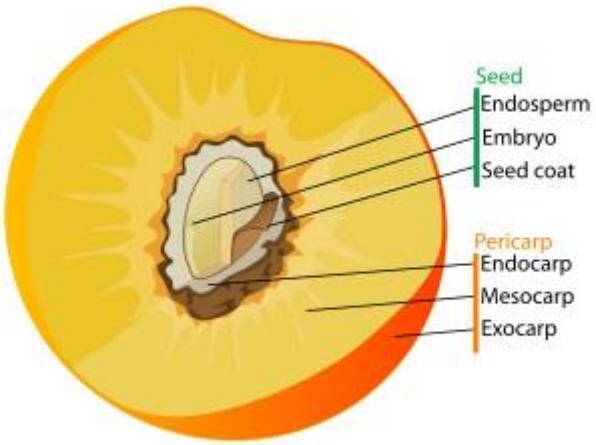

Fig.1.Coconut

Coconut is used for many purposes, for example – making ornaments and planting for decorative purposes. Coconut water is beneficial to our health as for refreshment, hydrating drink with lots of minerals, sugar and electrolyte (energy drink). It possesses anti-ageing and anti-carcinogenic properties. Coconut plant is also used to prepare alcohol, vinegar, timber, thatching material strips etc. Coconut fibres are used for making house wares such as utensil brushes, baskets, brooms mats, rope, caps and other articles. The most important product of coconut palm is oil, which is used for cooking, illuminating, for making soaps and cosmetics etc [1].

The coconut husk is mainly used in factories. Most part of husks are thrown away as domestic waste. From this waste environmental pollution increases and the problem of disposal of husk waste arises. So, we are trying to minimise the environmental pollution causes due to coconut husk by finding out their phytoconstituents and biological activities. Plenty of studies had been done on coconut leaves, water, and oil. But limited studies are available on the mesocarp of coconut. After research survey, we have planned to isolate phytochemicals from fresh mesocarp and evaluation of antioxidant activity of extract and isolated phytochemicals.

Materials and Methods

Materials and Instruments

Petroleum ether, Fehling's solution A and B, Biuret Reagent, Benedict Reagent, Molisch Reagent, Seliwanoff's Reagent, Millon's Reagent, Dragendroff's Reagent, Mayer's reagent, Ammonia, Lead acetate, Magnesium turning, and aluminium chloride was purchased from Loba Chemie Laboratory Reagent and Fine Chemicals Pvt. Ltd India, Chloroform and Acetone purchased from S. D. Fine Chemicals Pvt. Ltd., Mumbai (India), and methanol from Merck Specialities Pvt. Ltd., Mumbai (India). Ethyl acetate was purchased from Spectrochem Pvt. Ltd., Mumbai (India). Distilled water were prepared in quartz triple distilled water apparatus. While commercially grade Ethanol was purified by refluxing and distilled out over dry calcium oxide. Wagner's reagent, sulphuric acid, sodium hydrogen carbonate and sodium acetate were purchased from Fisher Scientific India. Potassium sodium tartarate was purchased from Finar Chemicals, India. Sodium hydroxide and

ninhydrin reagents were purchased from Sisco Research Laboratories Pvt. Ltd. (SRL), India. Olive oil was obtained commercially from local market and used without purification. All the aqueous solutions were prepared in quartz triple distilled water.

pH of different crude coir extract solutions was measured using pH meter of make Elico Li 120. Also, biological activities of crude extract were measured using Agilent Technologies Cary 60 UV-Visible Spectrophotometer.

Collection of plant

The plant material coir or mesocarp from coconut fruit (*Coccus nucifera)* was selected for the study. The coir was collected in May 2017, from street hawkers from the local market of Jalgaon, (India). The coir or mesocarp was identified and authenticated by Prof. K. S. Vishwakarma, School of Life Sciences, Kavayitri Bahinabai Chaudhari, North Maharashtra University, Jalgaon, (India).

The exocarp from the fruit was peeled off and the coir was crushed using a household mixer grinder. The extract was obtained using the Soxhlet apparatus by employing various solvents as per polarity, viz. petroleum ether, Ethyl acetate, Chloroform, Acetone, Methanol, Ethanol and Distilled water [5]. The extraction was carried out at different temperatures depending on the extraction solvent.

Physiochemical Parameters

- **Determination of Percentage Yield and pH**

The percentage yield of the mesocarp was determined by the methods described in the pharmacopoeia. Mesocarp when extracted with different solvents, we can measure its constituent in different solvents which is the indication of the extent of the polar, non-polar or medium polar component present in the husk. Also, the pH of the extract in different solvents gives information about the phytoconstituent which are acidic or basic [6].

Fresh mesocarp from *Coccus nucifera* was crushed and weighed. After weighing, it was subjected to Extraction by Soxhlet apparatus. After completion of the extraction process, solvents were evaporated, and crude extracts were obtained.

This crude extract was weighed and the per cent yield against fresh coir was calculated using the following formula [5].

$$\% \text{ Yield } = \frac{\text{Wt.of Extract}}{\text{Wt.of Fresh Coir taken}} \text{ x } 100$$

The pH of each extract of fresh coir was measured using Elico Li 120 pH meter after calibrating the pH meter using 7.0 and 9.2 buffer solutions. And the result for each solvent was reported.

- **Determination of Ash Content**

The Ash content of coconut coir was determined as Total Ash, Acid Insoluble Ash, and Water-Soluble Ash.

Total Ash

After a maceration of dried coir of *Coccus nucifera* at 500–600^{0}C for two hours in a muffle furnace, the total ash value of coir was calculated using the following formula for determination of inorganic matter form coir [6, 7].

$$\text{Total Ash } (\%\text{w/w}) = \frac{\text{Wt.of Ash}}{\text{Wt.of Sample}} \text{ x } 100$$

Acid Insoluble Ash

The 2 g total ash obtained from coir was mixed with 25 mL 2M HCl and boiled for 5 min in a silica crucible and filtered through ashless filter paper Whatman No. 41. The dried residue was again burst into muffle furnace at temperature 500–600^{0}C. After cooling in the desiccator for 30 min the white ash was weighed. The acid-insoluble ash was calculated as [6, 7].

$$\text{Acid Insoluble Ash } \left(\% \frac{\text{w}}{\text{w}}\right) = \frac{\text{Wt.of Ash}}{\text{Wt.of Sample}} \text{ x } 100$$

Water Soluble Ash

Again 2 g of total ash obtained from the coir was dissolved with 25 mL distilled water and boiled for 5 min. After filtration through ashless filter paper Whatman No.41, the insoluble residue was successively washed with 5 mL distilled water and then ignited at 500–600^{0}C. Then it was cooled in a desiccator and after 30 min the white ash was weighed. The water-soluble ash is calculated as follows [6, 7].

$$\text{Water Soluble Ash } \left(\% \frac{w}{w}\right) = \frac{(\text{Wt.of Ash} - \text{Wt.of Insoluble Ash})}{\text{Wt.of Sample}} \text{ x } 100$$

- **2.4 Phytochemical Screening**

Different qualitative tests for the determination of secondary phytoconstituent such as reducing sugar, glycosides, phenols, terpenoids, steroids, alkaloids, flavonoids, tannins, proteins, and amino acids etc were taken by using semi-solid extract of coconut husk [5, 8, 9].

Carbohydrates [8, 9]

The carbohydrates are qualitatively analysed by using four different tests. The equal volume of crude coir extract was mixed individually with different reagent solutions such as

Reagent	Observation
Fehling's solution	yellow to brick red precipitate.
Benedict's Solution	the solution appeared red.
Molisch's reagent	purple coloured produced.
Seliwanoff's reagent	red-coloured solution.

Protein [3]

The protein was qualitatively analysed as a small portion of crude coir extract was added to the reagent and warmed in the water bath. Following results were obtained.

Reagent	Observation
Millon’s reagent	brick red precipitate dissolved in giving red solution.
Biuret reagent	pink-coloured solution

Amino acid [3]

The amino acids were detected in the presence of a ninhydrin solution. The crude coir extract was heated with a few drops of 5% ninhydrin in a water bath for 10 min. The purple-bluish colour appears the presence of amino acid was confirmed.

Steroids / Terpenoids [10]

The steroids/terpenoids were qualitatively analysed by the Salkowski reaction. In a test tube of 2 mL each, crude coir extract, chloroform and concentrated sulphuric acid were added and Shaked well. The chloroform layer appeared red, and the acid layer showed greenish-yellow fluorescence, indicating steroids was present. And if reddish brown colour persists between interfaces of two layers, then terpenoids were present.

Glycosides [10]

The glycosides were detected by the Keller Killani test. In this 2 mL extract was taken, glacial acetic acid, one drop of 5% $FeCl_3$ and concentrated sulphuric acid were added. The observation was if reddish colour appears at the junction of two liquids and the upper layer appears bluish green. The glycoside was present.

Flavonoids [10, 11]

Flavonoid content from the extract could be determined in four different ways.

i. In Shinoda Test, dry crude coir extract in 95% ethanol was taken in the test tube followed by a few drops of concentrated HCl and 0.5 g magnesium turning. The appearance of orange, and red to purple colour indicates the presence of flavonoid.

ii. In the Aluminium Test, a few drops of 1 % of Aluminium solution were added to the extract. The yellow coloured indicated the presence of flavonoids.

iii. In aqueous filtrate of extract dilute ammonia solution and concentrated sulphuric acid were added. Yellow coloured appeared to the solution which on standing disappeared indicated the presence of flavonoids.

iv. The dry, powdered extract was heated with ethyl acetate over a steam bath for 5 min. After filtration, the filtrate was shaken with dilute ammonia. Yellow colouration was a sign of the presence of flavonoids.

Alkaloid [11–13]

The alkaloids were detected qualitatively using four different reagents. The dry crude coir extract was dissolved in dilute hydrochloric acid and filtrate was collected.

Reagent		Observation
Mayer's reagent	-	yellow-coloured precipitate
Dragendroff's reagent	-	orange-coloured precipitate
Hager's reagent	-	yellow precipitate observed.
Wagner's reagent	-	reddish brown precipitate obtained.

Tannins [10]

Tannins present in the crude extract were qualitatively analysed. In the extract solution, a few drops of acetic acid were added. Red colouration indicates that tannin was present.

Saponins [10, 11]

The saponins were qualitatively analysed using two different tests.

i) In Foam Test, the dry extract was shaken with water. If the foam was persisting for about 10 mins, then Saponins was present.

ii) The 2 g of dry extract was boiled with distilled water and filtered. To this filtrate, a few drops of olive oil were added and shaken vigorously. Emulsion gets formed. This indicates that Saponins was present.

Phenols [9, 11, 12]

Ferric chloride solution and 10% lead acetate solution were added to the crude coir extract. The presence of phenols was confirmed as a bluish-black colouration and milky white precipitate appeared respectively.

Reagent		Observation
$FeCl_3$Test	-	Bluish black colouration
Lead acetate Test	-	milky white precipitate

- ***2.5 Total Phenolics Content (TPC)***

The total Phenolic content of the crude coir extract was determined by using Folin–Ciocalteu reagent against standard gallic acid. Various concentrations of standard gallic acid and coir extract from different solvents were prepared ranging from 0.1 mg/mL to 10 mg/mL (as 0.1, 0.5, 1.0, 2.5, 5 and 10 mg/mL). To each 0.1 mL of gallic acid solution 0.5 mL distilled water and 0.1 mL Folin-Ciocalteu reagent was added. The reaction mixture was allowed to stand for 6 minutes. Further, add 1 mL of 7% sodium carbonate and 0.5 mL of distilled water. The reaction mixture was kept aside for 90 minutes. The absorbance was measured at 760 nm using a spectrophotometer. The same procedure was repeated for each coir extract from different solvents for different concentrations [5, 17–19].

- ***2.6 Total Flavonoids Content (TFC)***

Total flavonoid content was calculated by using Quercetin as a standard against the total flavonoid content of the crude extract. The dilutions of quercetin having concentrations 0.1, 0.5, 1.0, 2.5, 5.0 and 10.0 mg/mL were prepared in methanol. 0.1 mL of each dilution was mixed with 0.5 mL of distilled water and 0.1 mL of 5% sodium nitrate. Then allow to stand for 6 min.

Then 0.15 mL 10% aluminium chloride was added and again allowed to stand for 5 minutes. 1M 0.2 mL sodium hydroxide was added to this mixture and absorbance was recorded at 510 nm. The same procedure was carried out for crude coir extract. Total flavonoid content was calculated as quercetin equivalent (mgQE/g) from graph [5, 16–18].

- ***2.7 Antioxidant Activity by DPPH Method***

The antioxidant activities of different extracts of coconut husk in a different solvent were determined using the 2, 2–dipheny-1-pecryl hydrazyl (DPPH) method and ascorbic acid were used as standard. Using suitable solvents different concentrations of crude extract were prepared as 50, 100, 200 and 500 ppm. 2.9 mL extract solution was taken in a test tube and 0.1 mL 0.1 millimole DPPH solution in methanol was added. Allow to stand for half an hour. DPPH scavenging activity was recorded at 517 nm using Agilent Technologies Cary 60 UV-Visible Spectrophotometer [14–17].

- ***2.8 Antioxidant Activity by H_2O_2 Method***

The ability of coir extract to scavenge hydrogen peroxide was determined spectrophotometrically. 1mM H_2O_2 solution in 50 mM phosphate buffer having pH 7.4 was prepared. Extract of crude coconut husk having suitable solvent with concentrations 50, 100, 150, 200, 250 and 300 µg/mL were prepared. Then 0.2 mL of extract sample solution was added to 0.6 mL of 50 mM phosphate buffer. Then 1.2 mL H_2O_2 solution was added. The mixture was vigorously stirred. The absorbance of each test solution at 230 nm after 10 minutes against blank was measured. The blank solution is without an extract sample. The per cent inhibition was calculated using the formula [16].

$$\% \text{ Inhibition} = \left(1 - \frac{Abs.of\ Sample}{Abs.of\ Control}\right) \text{x } 100$$

Result and Discussion

- **Physicochemical Characteristics**

Determination of pH and Percentage Yield

The percentage yield of each crude extract was calculated and presented in Table No. 1. It indicates the amount of active constituent of the plant parts present in polar and non-polar solvents. This method can be employed where there is no biological assay possible. The pH of each crude extract indicates that the extracted Phyto-constituents are acidic or basic [5, 6].

Table 1. Physico-chemical parameter

Sr. No.	Solvent	Colour of Extract	Yield (%)	pH
1	Petroleum Ether	Milky white	0.27	8.66
2	Ethyl acetate	Wine red	1.90	7.94
3	Chloroform	White	0.61	6.88
4	Acetone	Wine red	6.51	7.34
5	Methanol	Wine red	1.50	8.30
6	Ethanol	Wine red	1.34	3.69
7	Distilled water	Wine red	8.71	3.73

Ash Content

Parameters such as total ash, acid-insoluble ash and water-soluble ash play important roles in determining the purity of the crude extract. These parameters are mainly measuring silicates, carbonates, phosphates, and silica that are formed from the ash of plant tissue. However, some carbonates may convert to oxide at very high temperatures (above 600^0C). The total ash, acid-insoluble ash and water-soluble ash values are 3%, 2.41% and 4.8% respectively [6, 7, 15]. These values also indicated whether the extract can be used as an herbal supplement.

Table 2. Result for Ash Values of Extract

Sr. No.	Coir Extract	Observations (in %)
1	Total Ash	3
2	Acid Insoluble Ash	2.41
3	Water Soluble Ash	4.8

- **3.2 Phytochemical Screening**

The different functional group present in crude extract was carried out using aqueous and non-aqueous solvents, detected phytochemicals are summarised in Table No. 3 [5]. Carbohydrates, proteins, terpenoids, flavonoids, and alkaloids are present in almost all extracts, while tannins, saponins and phenols are present up to some extent in different extracts of coconut coir.

Table 3. Phytochemical Screening

Sr. No.	Phytoconstituent	Pet Ether	Ethyl Acetate	Chloroform	Acetone	EtOH	MeOH	H_2O
1	Carbohydrates	(-)	(-)	(+)	(+)	(+)	(+)	(+)
2	Proteins	(-)	(-)	(-)	(+)	(+)	(+)	(-)
3	Amino Acids	(-)	(-)	(-)	(-)	(-)	(-)	(-)
4	Steroids	(-)	(-)	(-)	(-)	(+)	(-)	(-)
	Terpenoids	(-)	(+)	(+)	(+)	(-)	(+)	(-)
5	Glycosides	(-)	(-)	(-)	(-)	(-)	(-)	(-)
6	Flavonoids	(-)	(+)	(+)	(+)	(+)	(+)	(+)
7	Alkaloids	(+)	(+)	(+)	(+)	(+)	(-)	(+)
8	Tannins	(-)	(-)	(-)	(-)	(+)	(+)	(+)
9	Saponins	(-)	(-)	(+)	(-)	(+)	(+)	(+)
10	Phenols	(+)	(-)	(-)	(-)	(-)	(+)	(-)

(+) = present; (-) = absent

- **3.3 Total Phenolics Content (TPC):**

The optical density of standard reference gallic acid was measured and tabulated in Table No. 4. Fig No. 2 shows the curve and regression equation of gallic acid. Based on a curve and regression equation the total phenolic content of the extract was determined as gallic acid equivalents per gram (mgGAE/g) of dry extract of coir in different solvents. Crude coir extract of coconut in ethanol, methanol and distilled water extract contained 8.87 mg GAE/g, 7.25 mg GAE/g and 359 mg GAE/g. Distilled water shows the highest values for total phenolic content [5, 17, 18]. This indicates that the crude coir contains good amounts of phenolic content which are responsible for the antioxidant activity of the plant.

Table 4. Absorbance of Gallic acid

Sr. No.	Concentration	O. D. of Gallic acid
1	0.1	0.0209
2	0.5	0.0221
3	1	0.0245
4	2.5	0.0258
5	5	0.0261
6	10	0.0297

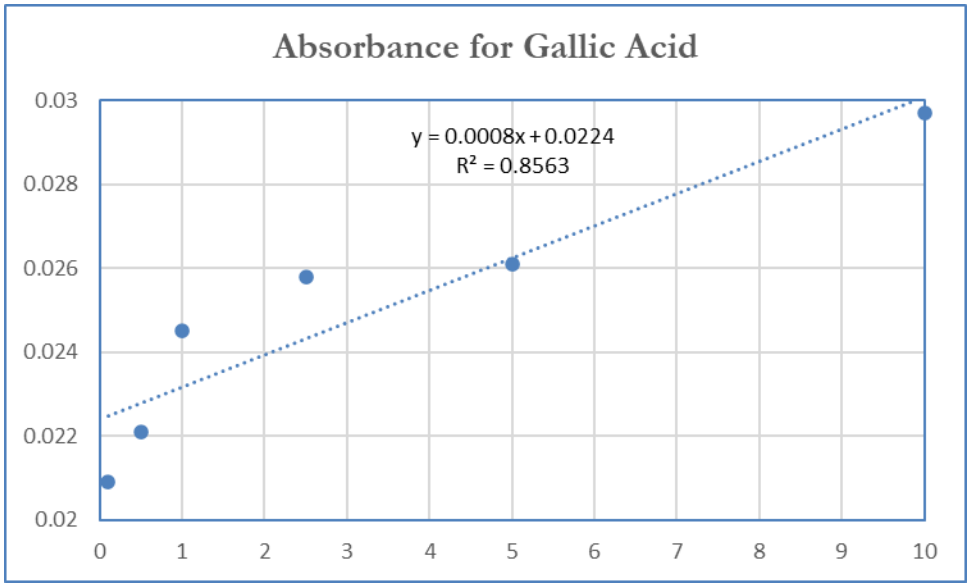

Fig. 2. Plot of Concentration of gallic acid

- **3.4 Total Flavonoids Content (TFC):**

Table No. 5 shows the absorbance of quercetin and Fig No. 3 shows the curve and regression equation from which total flavonoid content was estimated. In the present investigation, we found the amount of Total flavonoid Content. It is higher in ethyl acetate extract having a value of 30.82 mg QE/g and lower in ethanol extract having a value of 13.64 mg QE/g [5, 16–18]. These results reveal that the good number of flavonoid contents present in fresh coir is in noticeable amount and can be further responsible for the anti-diabetic activity.

Table 5. Absorbance for Quercetin

Sr. No.	Concentration	O. D. of Quercetin
1	0.1	0.0639
2	0.5	0.0862
3	1	0.1250
4	2.5	0.2717
5	5	0.3593
6	10	0.6253

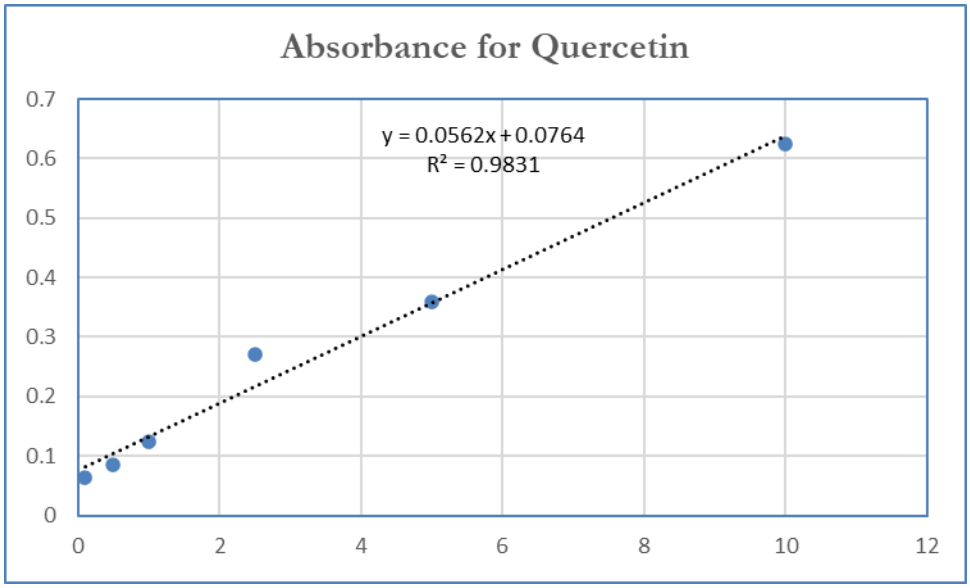

Fig. 3. Plot of Concentration of quercetin

- **3.5 Antioxidant Activity by DPPH**

All results obtained were carried out in triplicate. The activity of natural antioxidants present in fresh mesocarp extract to reduce 2, 2 – diphenyl – 1- pecryl hydrazyl would be measured in a UV-visible spectrophotometer at 517 nm. The ascorbic acid which is a good antioxidant used as a reference. The results obtained

show the percentage of reduction of extract as compared to ascorbic acid. The % Inhibition activity of DPPH free radicals was calculated from the decrease in absorbance of the sample extract. The % inhibition values of the DPPH assay done for different solvent extracts are tabulated below table. According to the result, and comparing it with other results, crude extract of coconut coir in pet ether and ethanol shows significant antioxidant activity with per cent inhibition of 89.77% and 86.84% respectively. While ethyl acetate, acetone, and distilled water extract exhibit moderate scavenging activity with per cent inhibition of 64.75%, 66.93% and 56.52% respectively [20, 21].

Table 6. Per cent Inhibition by DPPH

Concentration (in ppm)	AA	PE	EA	Acetone	Chloroform	EtOH	MeOH	DW
50	78.73	52.46	66.75	58.98	11.5	48.77	16.18	76.71
100	82.95	62.97	66.15	73.22	5.27	86.67	6.09	83.25
200	92.38	63.93	64.96	77.35	6.3	86.13	8.17	83.7
500	97.83	89.77	64.75	66.93	7.93	86.84	1.71	56.52

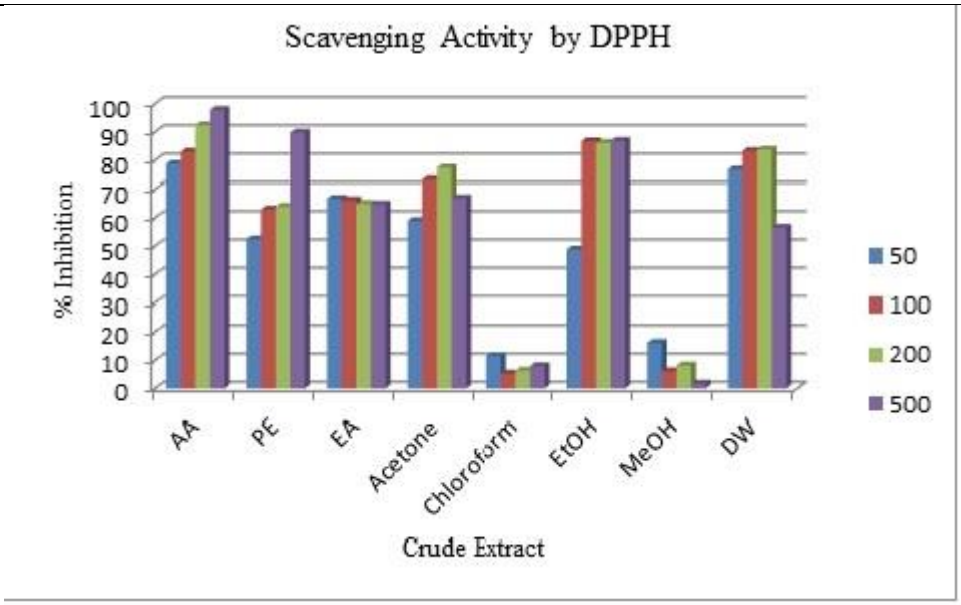

Fig. 4. Plot of scavenging activity by DPPH

- **3.6 Antioxidant Activity by H_2O_2**

Hydrogen peroxide is a weak oxidising reagent and can inactivate some enzymes. Hydrogen peroxide reacts with Fe^{2+} and Cu^{2+} ions resulting in the formation of hydroxyl radicals which can exert toxic effects. The scavenging assay by peroxide can be measured by calculating the percentage inhibition of extract against standard reference ascorbic acid. The result clearly shows that the crude coconut coir extract in ethanol showed maximum per cent inhibition by the hydrogen peroxide method having a value of 97.42% at 300 µg concentration. Crude coconut coir extract in acetone and chloroform showed moderate per cent inhibition and calculated inhibition values are 72.81% and 72.21%, while water extract of coconut husk showed minimum inhibition activity with a value of 37.93% per cent at 300 µg concentration [16].

Table 7. Per cent Inhibition by H_2O_2

Concentration (in ppm)	PE	EA	Acetone	Chloroform	EtOH	MeOH	DW
50	64.35	152.37	119.6	67.91	114.4	432.9	66.07
100	-23.28	176.5	1.73	4.46	108.3	-422.1	12.77
150	-301.2	223.2	75.4	81.92	113.2	474.6	34.55
200	264.4	1041.2	5.51	0.59	103.2	255.4	9.08
250	176.99	78.73	91.48	7.54	101.7	570.9	81.83
300	2.389	38.25	72.85	72.21	97.42	-60.78	37.93

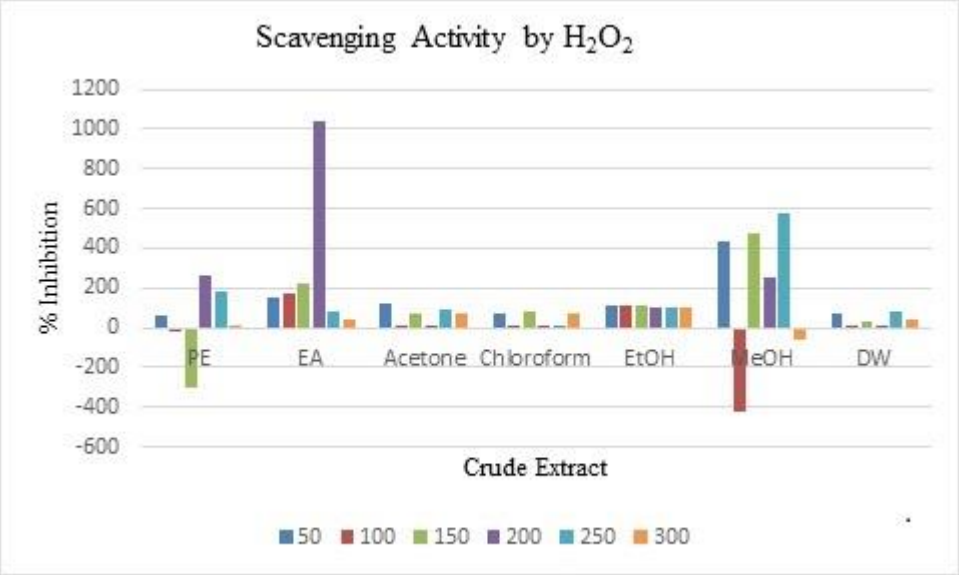

Fig. 5. Plot of scavenging activity by H_2O_2

Conclusion

Based on the phytochemical screening of coconut plants, in aqueous and non-aqueous extract various phytochemicals were identified viz vitamins, flavonoids, terpenoids, minerals etc. All these phytochemicals are important for various food materials as well as pharmaceutical preparations. These phytochemicals show different biological activities. Coconut husk is very popularly used in industrial productions, if we use the phytochemicals produced from it, we can be able to find alternative and cheap sources for the food as well as pharmaceutical industries. From the TFC and TPC analysis, the coir extract should be subjected to drug discovery.

The results are encouraging and may open newer avenues for herbal medicine from coconut and its related products. In the future, more attempts will be made to evaluate other activities of extract. In conclusion, aqueous and non-aqueous extracts may be used as herbal supplements for better health.

Acknowledgement

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