



## Recovery of polyphenols from agro-food byproducts: Coconut shell and groundnut hull

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### ABSTRACT

Agro-food byproducts such as coconut shell (*Cocos nucifera* L.) and groundnut hull (*Arachis hypogea* L.) have been considered as waste materials and hence if they are not handled properly, they would cause severe environmental problems. One of the interesting approaches is to use them as natural antioxidants for the development of nutraceuticals. Hence, in the present study we have focused on the recovery of polyphenol compounds from coconut shell and groundnut hull using different treatments and evaluated their antioxidant potential. Polyphenol compounds were isolated using solvent extraction with / without shaking and acid / alkali hydrolysis and their total phenolic content was analyzed using Folin-Ciocalteu method and antioxidant power using DPPH radical scavenging activity. Alkali hydrolysis was found to be efficient in the recovery of total phenolic compounds of coconut shells (950.25 mg GAE/100 g) as well as ground nut hull (94.00 mg GAE/100 g), but the antioxidant power was higher in acid hydrolysis of coconut shells (82.59%) and groundnut hull (73.65%). Both coconut shell and ground nut hull exhibited 57.1 and 70.0% inhibition of lipid peroxidation, respectively in model food system. Between the two investigated agro-food byproducts, groundnut hull revealed maximum inhibition of lipid peroxidation and hence it could be considered as a potential source of antioxidants for the development of nutraceuticals.

**Keywords:** Byproducts, Coconut, Groundnut, Polyphenols, Antioxidant.

### INTRODUCTION

Food spoilage is the process in which food deteriorates and its quality and edibility become reduced. Harvested foods decompose from the moment they are harvested due to attacks from enzymes, oxidation and microbes<sup>1</sup>. Infestations (invasions) by insects and rodents also account for huge losses in food stocks. Lipid peroxidation is the oxidative degeneration of lipids, in which free radicals remove electrons from the lipids and results in lipid damage. Lipid peroxidation mostly occurs in unsaturated fatty acids as they contain more number of double bonds. Reactive oxygen species such as superoxide, hydroxyl and lipid peroxy radicals are the initiators of lipid peroxidation.<sup>2</sup> Synthetic antioxidants include butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) and propyl gallate are added to food to prevent undesirable deterioration.

However, synthetic antioxidants cause several health problems including cancer and hence there is a need for alternative natural food antioxidants<sup>2</sup>. Naturally occurring antioxidants include vitamin A, vitamin E and vitamin C. These may play a significant role in the prevention of food spoilage, but their meager quantity in foods is not sufficient to prevent the spoilage in long term storage conditions<sup>3</sup>. Hence, the polyphenolic compounds from cost-effective sources like agro-food byproducts and applied in food preservation<sup>2</sup>. In this context, we have selected coconut shell and groundnut hull as a natural source of antioxidants and optimized for the recovery of poly phenols to apply in food preservation.

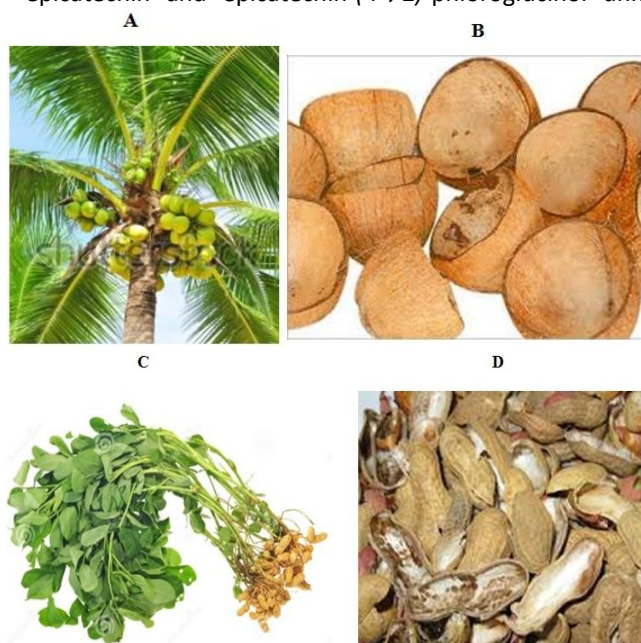
The coconut (*Cocos nucifera* L.) tree belongs to the family of Arecaceae, which is grown in the tropical regions of the world (Figure 1). It sustains the livelihood of millions of people in coastal regions of tropics. There are two main distinct varieties of coconut such as tall and dwarf. Coconut palms have been called the "tree of life" because of the large number of uses. Coconut ranks seventh most important vegetable oil crop. Coconut trees are grown mainly for high oil content, which is utilized in food and other purposes. From 1980 to the present year, total world coconut productivity has increased significantly from 35 to 50 million tonnes<sup>6</sup>. India is the third largest producer of coconut in the world, cultivates 9 million tonnes of coconut trees in a vast area of 2 million hectares<sup>6</sup>. People from many diverse cultures, languages and religions are scattered around the globe have revered the coconut as a valuable source of both food and medicine.

Coconut water from the tender young fruit is a delicious and nutritious beverage. Coconut provides a nutritional source of quick energy. Coconut oil has been described as the healthy oil. Coconut has a caloric value of 17.4/100 g. The coconut water contains nicotinic acid B3 (0.64 µg/mL), pantothenic acid B5 (0.52 µg/mL), biotin (0.02 µg/mL), riboflavin (<0.01µg/mL), trace amount of thiamine B1 and pyridoxine B6 (USDA, 2008). Coconut water contains sugars, sugar alcohols, vitamin C, folic acid, free amino acids, phytohormones and growth promoting factors. The readily fermentable sugars present in coconut water made it to be used in



production of coconut vinegar, wine and toddy. Catechin, epicatechin and epicatechin-(4→2)-phloroglucinol units

are present in high amounts in coconut<sup>7</sup>.



**Figure 1:** Morphology of coconut (A) and groundnut (B) plants and their byproducts (C & D)

Coconut palm byproducts such as husks, shells, leaves and the stem find many applications. Coconut production gives rise to coir as the by-product, whereas the husks are considered as waste and left behind as mulch, or sometimes used as fertilizer due to its high potash content. It is estimated that the production of coir pith in India is about 7.5 million tonnes per year. Coconut husk is the mesocarp of coconut and a coconut consists of 33–35% of husk. Husks are composed of 70% pith and 30% fiber on a dry weight basis. The world production of coir fibers ranges between 5 and 6 million tonnes per year. Coconut husk has a good fibrous structure and its coir fibers are used in manufacture of mattresses, rugs and ropes. As it contains lignin, cellulose and hemi cellulose, coconut husk can be used as a good potential energy source. It has been known for some time that this antimicrobial metabolite is present in the coconut<sup>8</sup>. The husk fiber decoction has been used in northeastern Brazil traditional medicine for treatment of diarrhea and arthritis<sup>9</sup>. It produces more smoke so it can be used as a fuel in indirectly-fueled kilns<sup>10</sup>.

Coconut shells are used in several ways such as home decorative articles, lamp shades and frames. The powdered form of coconut shell is used as the main component in the manufacture of glue. Apart from its home uses such as cooking, ironing, coconut shell is also used as a substitute in the bunker oil in boiler operations. Coconut shells are also used in manufacture of charcoal by pyrolysis of the shells in the absence of air at 600 C.<sup>10</sup> Further processing of coconut shell-charcoal by treating it with oxidizing agents such as steam and carbon-di-oxide results in the formation of

activated carbon. Activated carbon has high adsorptive capacity because of its high surface area. Because of its small pore structure and high mechanical strength, this can be used in gas adsorption<sup>10</sup>.

The groundnut (*Arachis hypogea* L.) originated from south America. The term groundnut is used in most countries of Asia, Africa, Europe and Australia while in North and South America it is referred as peanut. It is a legume crop and not related to other nuts. India is the second largest producer of groundnuts in the world (Figure 1). Indian groundnuts are available in different varieties: Bold or Runner, Java or Spanish and Red Natal. Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra, Rajasthan, Madhya Pradesh, Orissa, and Uttar Pradesh are the major states in India where groundnut is cultivated in large quantity. The country has exported 7,08,386.24 MT of groundnuts for the worth of Rs. 4,675.35 Crores during the year 2014-15<sup>11</sup>.

The genus groundnut belongs to a family of Fabaceae. This genus is morphologically well defined and distinguished from other genera by having a peg and geocarpic reproductive growth. Although botanically a legume, it is consumed as a nut and marketed internationally as an oil-seed crop due to its high oil content. Groundnut flour is used to replace part or all of wheat flour or corn meal in making of breads and other bakery products. Groundnut oil has been used to make cheese products. Groundnuts are rich in essential nutrients (USDA nutrient data). In a 100 g serving, groundnuts provide 570 calories and are an excellent source (defined as more than 20% of the Daily Value, DV) of several B vitamins, vitamin E, several dietary minerals, such as manganese (95% DV), magnesium (52% DV) and phosphorus (48% DV), and dietary fiber (right table). They also contain about 25 g protein per 100 g serving, a higher proportion than in many tree nuts. Groundnuts are used in making snacks and other food items.

De-oiled groundnut cake is used to replace fish meal as it contains proteins. Several authors<sup>12-16</sup> have reported that groundnut skins, hulls and roots have high levels of poly phenols with demonstrated antioxidant properties. The development of more efficient methods for extracting antioxidant compounds from groundnut skins is needed in order to increase commercial appeal. Groundnut shell have been used as fuel for moving boilers in manufacturing processes, soil conditioners, carriers for chemicals, activated carbon and fertilizer. Groundnut shells are often used as cattle feed. Groundnut shell is use in composting of wet materials, for waste water treatment, plastic, wardrobes. Groundnut shell is a carbonaceous, fibrous solid waste which encounters disposal problem and is generally used for its fuel value. Proanthocyanidins isolated from

the water-soluble fraction of peanut skins exhibited antioxidant activity<sup>13</sup>.

The total cellulose content in groundnut hull is found to be 65.5-79.3% whereas other components such as Hemicelluloses (10.1%), carbohydrates (10.6-21.2%), Proteins (4.8-7.5%), Calcium (0.24-0.27%) and Minerals (4.3%). Groundnuts contain bioactive phytochemicals including the widely studied polyphenols such as daidzein, genistein and trans-resveratrol. A study found that phenolic compounds extracted from peanut skins could significantly reduce the oxidation of meat products and extend their storage stability<sup>17</sup>. Phenolic compounds extracted from defatted peanut skins presented a greater protective effect against the haemolysis of red blood cells than ascorbic acid under *in vitro* conditions<sup>18</sup>.

The byproducts from coconut and groundnut have been considered as waste products and hence if they are not handled properly, they would cause environmental problems. One of the interesting approaches to utilize these byproducts is isolation of natural antioxidants. Hence, in the present study we have focused on isolation of poly phenol compounds from coconut shell and groundnut hull using different treatments and evaluated their antioxidant potential. Further application of poly phenol extracts from these agro-food byproducts in food preservation was investigated using antioxidant and lipid peroxidation inhibition potentials in model food system.

## MATERIALS AND METHODS

### Sample collection

Coconut shell and groundnut hull samples were collected from Kumbakonam, Thanjavur District during month of December 2015. The samples were dried under shaded condition and powdered in the grinding mill at Pharmacy unit of CARISM, SASTRA University. The samples were stored in polythene bag and used for further experiments.

### Preparation of extracts

The samples were treated under different conditions (solvent extraction with / without shaking, acid and alkali hydrolysis) at different timings to optimize the recovery of polyphenols. For solvent extraction, we used 100 ml of 70% ethanol by taking 10g of powdered sample in a conical flask and kept at room temperature for 6 h. Then the contents were filtered using filter paper and the samples were drawn at every hour. The solvent extraction was performed at two different conditions (with and without shaking). For shaking, magnetic stirrer was used at 650 rpm. In acid hydrolysis, 10g of samples were mixed with 100ml of 2% Hydrochloric acid, whereas in alkaline hydrolysis 2% sodium hydroxide was used. Both acid and alkali hydrolysis were performed at room temperature, contents were filtered and the samples were drawn at every one hour and

analyzed for total phenolic content and antioxidant activity.

### Total phenolic content

Extract (10  $\mu$ l) was taken in a micro plate wells and added with 25 $\mu$ l of Folin's reagent and 230  $\mu$ l of sodium carbonate (4.4%)<sup>19</sup>. Then the micro plate was incubated for 30 minutes in the dark and read at 750 nm in the plate reader (Make: Biotek, model: Epoch). The Total phenol content (TPC) for the samples can be calculated using the formula: (absorbance - c / m x dilution factor) where c is the constant obtained from galic acid curve and m is the slope of galic acid curve. The formula for calculating TPC is  $y = 0.002x + 0.182$  and the value of  $r^2 = 0.994$ . Total phenol yield (TPY) is calculated using the formula: (TPC x volume of extract / weight of sample x 100).

### Antioxidant activity

The DPPH assay was conducted to determine the antioxidant activity of the sample<sup>20</sup>. The DPPH reagent is prepared by dissolving 5mg of DPPH in 100ml of methanol. The assay is initiated by adding 10 $\mu$ l of the extract to the micro plate wells and 200 $\mu$ l of the DPPH reagent was added and incubated for 30 minutes in dark. The plates were read at 515nm in a multi-plate reader. DPPH activity is measured using the formula (Absorbance of blank - Absorbance of sample / Absorbance of blank x 100) and the results are expressed in percentage basis.

### Inhibition of lipid peroxidation

Peroxidation assay is normally conducted to determine the ability of extract to inhibit peroxidation in lipid food samples<sup>21</sup>. The groundnut kernel is used as the source of lipids. Dried and roasted kernels were collected and ground to fine powder. The sample (25 g) was extracted with 50 ml hexane and stirred for 30 min and filtered. The collected groundnut fat was treated with 2.5 ml of extract of coconut shell and groundnut hull, separately and boiled for 1 h at 100 C. A control was performed without extract while blank was not boiled. The contents were mixed with 30ml of acetic acid/chloroform (3:2 ratios), 0.5ml of saturated potassium iodide and kept for 2 min. Then, 30ml of distilled water followed by 0.5ml of starch was added and the contents were titrated against 0.01M sodium thio sulfate. The amount of thio sulfate consumed is noted and then calculations were performed for peroxide value (PV) using the formula (Titration value x 0.01 / Weight of food lipid sample) where 0.01 is the molarity of sodium thio sulfate. Lipid peroxidation inhibition is calculated for these samples using the formula (PV control - PV extract / PV control x 100).

### Purification and LC-MS analysis

The groundnut hull extract was made into slurry with silica and loaded on the stationary phase and separated by using various solvents (hexane, chloroform, ethyl





acetate, methanol, ethanol and water. Glass column (60 cm length, 3 cm dia) was packed Silica. Totally five fractions were collected with hexane, chloroform, ethyl acetate, methanol, ethanol. Among the five fractions, methanol fraction was found to contain higher content of total phenols. Hence, the methanolic fraction of groundnut hull was analyzed in LC-MS to identify the phyto chemical compounds.

UHPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with Column: Shim-pack XR-ODS III (100 x 2 mm, 2.2  $\mu$ m particle size) Column temp. 40°C. The mobile phase consisted of (A) 0.1% formic acid in water and (B) Acetonitrile. Both mobile phases were filtered through a cellulose nitrate filter, diameter 47 mm, pore size 0.45  $\mu$ m (Sartorius, Goettingen, Germany). After the gradient separation, the column was re-equilibrated for 5 min using the initial solvent composition. The flow rate was set to 1 mL/min. Samples were kept in amber vials at 4 °C in the auto sampler, and the injection volume was 5  $\mu$ L. The separation was performed at 25.0  $\pm$  0.1 °C. LC-MS/MS System (Make: Shimadzu Corporation, Kyoto, Japan, Model: LCMS 8040, Triple

Quadrupole) Ionization: ESI (Negative mode), Ion spray voltage: + 4.5 kV / – 3.5 kV, MRM: 427 MRM transitions (2 MRMs / compound) Dwell time 5 msec. / Pause time 1 msec. Ambient CDL Temperature: 250°C, Block Temperature: 400°C, Detector voltage: 1.3 kv, Nebulizer Gas flow: 1.5 L/min, Drying gas: 10 L/min Detection. The resulting chromatogram and mass spectra details are given below.

## RESULTS AND DISCUSSION

### Total phenol content

Phenols are a class of chemical compounds consisting of a hydroxyl group bonded directly to an aromatic hydrocarbon group. Recently published reports indicate that the poly phenols are demonstrated to have strong antioxidant activities, such as scavenging activities against DPPH radicals, superoxide radicals and hydroxyl radicals<sup>22</sup>. Since free radicals are discussed to play a key role in the pathology of diseases such as cancer, atherosclerosis or inflammatory diseases<sup>23</sup>, the supply of antioxidants is of high importance for a healthy life.

**Table 1:** Recovery of phenolic compounds from coconut shell and groundnut hull during different conditions at different time intervals

Extraction time (hours)	Total phenolic content of coconut shell (mg GAE /L)			
	Solvent extraction without agitation	Solvent extraction with agitation	Acid hydrolysis	Alkali hydrolysis
1	415.75 $\pm$ 46.32	317.50 $\pm$ 54.45	131.25 $\pm$ 8.84	761.25 $\pm$ 15.20
2	418.00 $\pm$ 2.83	330.25 $\pm$ 37.83	0	691.75 $\pm$ 19.45
3	444.75 $\pm$ 29.34	371.00 $\pm$ 51.62	115.00 $\pm$ 15.56	876.75 $\pm$ 73.19
4	602.00 $\pm$ 262.34	439.75 $\pm$ 49.14	122.75 $\pm$ 44.90	900.00 $\pm$ 0.71
5	476.25 $\pm$ 0.35	368.25 $\pm$ 47.02	106.00 $\pm$ 37.48	950.25 $\pm$ 11.67
6	499.00 $\pm$ 19.09	345.75 $\pm$ 15.20	127.25 $\pm$ 15.20	941.75 $\pm$ 171.47
	Total phenolic content of groundnut hull (mg GAE /L)			
1	91.75 $\pm$ 37.83	0	0	51.50 $\pm$ 19.09
2	5.50 $\pm$ 0.70	0	138.50 $\pm$ 16.26	63.75 $\pm$ 1.06
3	2.25 $\pm$ 0.35	0	0	92.50 $\pm$ 33.94
4	4.00 $\pm$ 5.66	0	0	83.50 $\pm$ 15.56
5	32.50 $\pm$ 5.66	0	0	94.00 $\pm$ 3.54
6	14.25 $\pm$ 0.35	0	0	76.50 $\pm$ 4.24

The phenolic content of coconut shell extract is high (602.00  $\pm$  262.34mg GAE /L) at 4<sup>th</sup> hour for solvent extraction without agitation, (439.75  $\pm$  49.14mg GAE /L) at 4<sup>th</sup> hour for solvent extraction with agitation, (131.25  $\pm$  8.84mg GAE /L) at 1<sup>st</sup> hour for acid hydrolysis and (950.25  $\pm$  11.67mg GAE /L) at 5<sup>th</sup> hour for alkali hydrolysis (Table 1). On the whole, alkali hydrolysis gives the maximum phenolic content (950.25  $\pm$  11.67mg GAE /L) at 5<sup>th</sup> hour (Table 1). Looking in the solvent extraction without agitation we decide that the maximum TPC occurs at 4<sup>th</sup> hour and then it reduces for the next two intervals. Similar kind of result is observed

in solvent extraction with agitation and acid hydrolysis. In the case of alkali hydrolysis, maximum TPC occurs at 5<sup>th</sup> hour and then it reduces in the next interval.

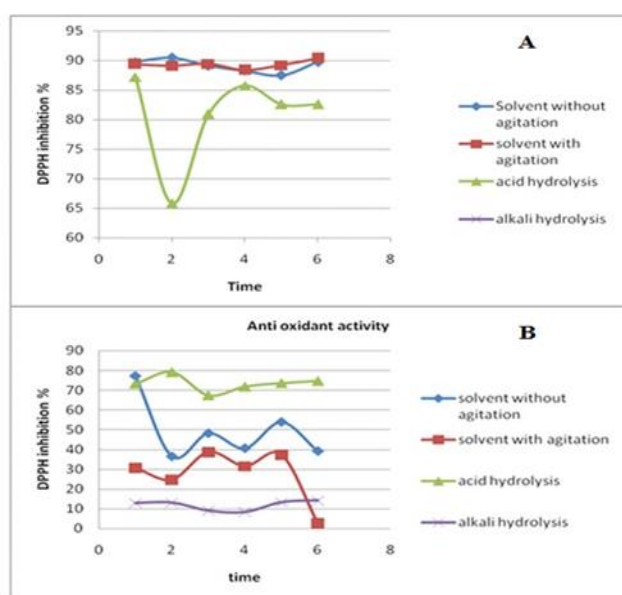
From the above obtained results (Table 1), it is clear that the phenolic content of groundnut hull is high (91.75  $\pm$  37.83) in the 1<sup>st</sup> hour in solvent extraction without agitation. In solvent extraction with agitation, there was no phenolic content though we repeated it twice. The phenolic content is high (138.50  $\pm$  16.26) at the 2<sup>nd</sup> hour in acid hydrolysis, (94.00  $\pm$  3.54) at 5<sup>th</sup> hour in alkali hydrolysis. On the whole, acid hydrolysis gives maximum phenolic content of 138.50  $\pm$  16.26. In the

case of solvent extraction without agitation, maximum TPC recovered at 1<sup>st</sup> hour and it reduces for the other time intervals. In the case of alkali hydrolysis, TPC obtained maximum at 5<sup>th</sup> hour and it reduces for the next interval. Here, we observe fluctuations in the results which may be due to manual errors or may be due to the impact of environmental conditions. Similarly, in a previous study, antioxidant properties of methanolic extract from peanut hulls was investigated<sup>24</sup>, and luteolin was identified as a major flavonoid in mature peanut to show high antioxidant activity<sup>25</sup>.

### Antioxidant activity

Antioxidant is molecule used to terminate the chain reaction which produce free radicals and cause damage to the cellular system<sup>12</sup>. The antioxidant inhibits the oxidation reaction that happens in any food sample and results in food spoilage. The phenol reacts with DPPH reagent and donates ions to convert the free radicals to stable atoms.

The DPPH inhibition percentage of the coconut shell extract prepared from different treatments are shown in Figure 2. Antioxidant activity of 90.51% was observed in the extract obtained from solvent extraction without agitation, 90.39% in solvent extraction with agitation, and 87.2% in acid hydrolysis. From the data, it is clear that the antioxidant activity of coconut shell was found to be maximum during solvent extraction without agitation. Antioxidant activity of groundnut hull extract 77.24% was seen in the solvent extraction without agitation, 38.66% in solvent extraction with agitation, 79.21% in acid hydrolysis and 14.19% in alkali hydrolysis. The antioxidant activity of groundnut hull was found to be maximum during acid hydrolysis.

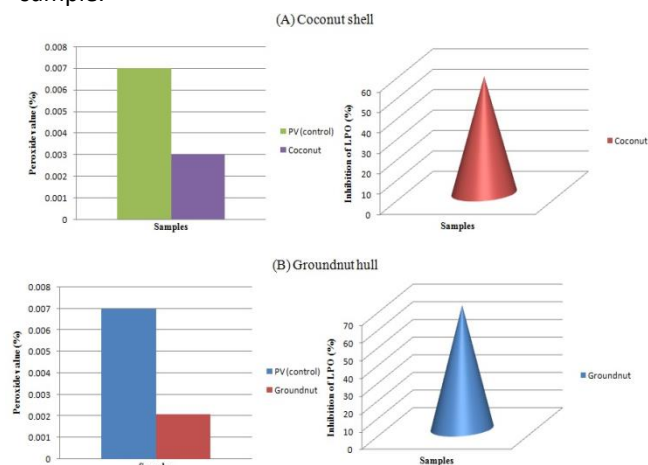


**Figure 2:** Effect of different extraction conditions on the antioxidant activity of coconut shell (A) and groundnut hull (B).

### Inhibition of lipid peroxidation

Anti-oxidants are substances capable of reducing free radicals and prevent them from causing cell damage<sup>26</sup>. Over the last decade, growing scientific evidence suggests that poly phenols used as antioxidants may protect cell constituents against oxidative damage and, therefore limit the risk of various degenerative diseases associated with oxidative stress<sup>27</sup>. Lipid peroxidation is the oxidative degradation of lipids. This process proceeds by a free radical chain reaction mechanism. If not terminated fast enough, there will be damage to cell membrane which consists of lipids in it<sup>28</sup>.

In the figures 3, we have shown the effect of coconut shell and groundnut hull extracts on the peroxide values of groundnut fat and the inhibition potential of lipid peroxidation. We have observed that the peroxide value is higher in the case of heated fat (control) and it is reduced when the coconut shell extract added to the sample. The lipid peroxidation in groundnut fat was inhibited by coconut shell extract (57.1%). We have observed that the peroxide value is reduced when the groundnut hull extract added to the sample. The inhibition of lipid peroxidation in groundnut fat sample by groundnut hull was 70%. Between the two samples, groundnut hull was found to be more effective in controlling the lipid peroxidation of high-fat food sample.

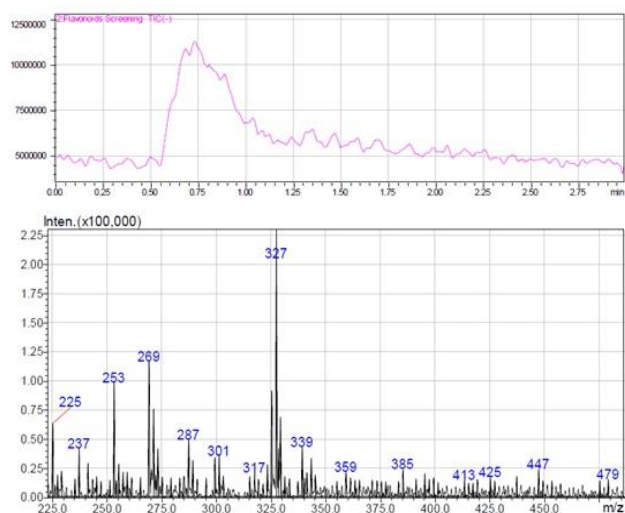


**Figure 3:** Peroxide value and lipid peroxidation inhibition potential of coconut shell (A) and groundnut hull (B)

### Purification and LC-MS analysis

Since, only groundnut hull extract was noticed to exhibit higher level of lipid peroxidation inhibition when compared to coconut shell, the groundnut hull extract was purified using column chromatography and eluted with different solvents of increasing polarity. Five fractions were collected with hexane, chloroform, ethyl acetate, methanol and ethanol. Among the five fractions, methanol fraction was found to contain higher content of total phenols (84.5 mg GAE / L). Hence, the methanolic fraction of groundnut hull

sample was analyzed in LC-MS to identify the phytochemical compounds. The major peaks observed in mass spectra were 328, 270, 254 and 226, which corresponds to the presence of phytochemicals such as Bergenin and Genistein (Figure 4).



**Figure 4:** LC-MS analysis of methanolic fraction of groundnut hull extract.

## CONCLUSIONS

In this study, we have focused on extracting polyphenols from coconut shell and groundnut hull. The results showed that coconut shell yields higher polyphenol content in alkali hydrolysis at 5<sup>th</sup> hour whereas groundnut hull in acid hydrolysis at 2<sup>nd</sup> hour. From the results obtained from DPPH assay, we get to know that maximum antioxidant activity is seen in solvent extraction without agitation in coconut shell and acid hydrolysis in groundnut hull. From these values, coconut shell shows greater antioxidant activity than groundnut hull. However, in lipid peroxidation inhibition assay, groundnut hull extract is found to be more effective in controlling the lipid peroxidation when compared to coconut shell extract. The phytochemicals such as bergenin and genistein were detected in the groundnut hull extract through LC-MS study, which might be responsible for the observed strong antioxidant effect. So we conclude that, agro-food byproducts of groundnut hull could be used in an efficient way as natural and cost-effective source of antioxidants in food preservation to prevent the food spoilage. Further, toxicity of the extracts should be evaluated using suitable *in vivo* models to advocate them as food preservatives in industries.

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