

Evaluation of Antioxidant and Antimicrobial Activities of Coffee Husk Extract

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Abstract:

The antioxidant and antimicrobial activity of 70% (v/v) aqueous methanolic extract of coffee husk was evaluated. Extracts of coffee husk were prepared by refluxing the dried coffee husk with aqueous methanol (70% v/v), water, methanol, ethylacetate and hexane. Maximum amount of polyphenols in coffee husk extract (CHE) were obtained from aqueous methanol (70% v/v). Total phenolic content were determined using folin ciocalteu's method. Antioxidant activity of CHE was assayed through invitro models such as antioxidant capacity by iron (111) reduction method, β -carotene-linoleate model system and free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Addition of 1000 ppm of CHE to refined sunflower oil and storage at room temperature ($22 \pm 3^\circ\text{C}$) for 80 days showed lower peroxide value (POV) and free fatty acid (FFA) content than control samples POV and FFA. Refined sunflower oil contains 100 ppm of butylated hydroxytoluene (BHT) and 1000 ppm of green tea polyphenols (GTP) and 1000 ppm of rosemary showed POV of 55.0, 112.07 and 109.9 meq oxygen/kg and FFA content of 0.87, 1.02, 1.06 mg KOH/g, respectively after 80 days of storage at room temperature. The minimum inhibitory concentration of CHE required against E.coli, S.aureus, S.typhi and S.enterica were 850, 800, 950, 1100 ppm respectively.

Key words:

Coffee Arabica, Coffee Husk Extract, Antioxidant activity, Antimicrobial activity.

1. Introduction:

India is one of the major coffee producers in the world with approximate annual production of 27.52×10^3 tones/year. It is grown in Western hill ranges of Southern peninsular of India, Coffee husk is a major agro-industrial residue obtained after removal of pulp and seed from fruit of coffee arabica and is estimated to yield 0.36 million tones/year (Sanjay Mande).

Major portion of coffee husk is available at the time of coffee harvest. This makes its collection easy but creates storage problem due to its low bulk density. Until recent past, the major application of coffee husk was to convert it into fuel and chemical feedstock through a variety of thermo chemical conversion process. Scrutiny of literature revealed that one methodology based on solid state fermentation (cultivation) to produce aroma (Soares, Christen, Pandey and Soccol, 2000; Medeiros, Pandey, Christen, Freitas, Fontoura and Soccol, 2001) citric acid (Carlos, Soccol, Luciana, Vandenberghe, Cristine Rodrigues and Ashok Pandey, 2006) and edible mushroom flammulina (Leifa, Pandey and Soccol 2001) has been reported. Coffee husk has no fertilizer value as it contains mostly lignocellulose but it contributes to the problem of environmental pollution. It contains organic solid residues such as caffeine, tannins and polyphenols (Fan, Pandey and Soccol 1999) and these have not been utilized so far. Polyphenols are important constituents of plants and they contribute directly to the antioxidant activity due to their excellent hydrogen donor property or have synergistic effects when used together with phenolic antioxidants (Pathirana, Shahidi and Alasalvar, 2006).

Antioxidants are compounds, which help to inhibit many oxidation reactions caused by free radicals. Free radicals are unstable molecules that include hydrogen atom, nitric oxide and molecular oxygen. They naturally occur in the human body as a result of chemical reaction during normal cellular processes. Reactive oxygen species (ROS) sometimes called as active oxygen species are various forms of activated oxygen, which include free radicals such as superoxide (O_2^-) and hydroxyl radical (OH), as well as non-free radical species such as hydrogen peroxide (H_2O_2) (Duthie, Duthie and Kyle, 2000). Spoilage of food due to bacterial and fungal infection has been a major concern for decades and it causes a considerable loss worldwide. The demand for non-toxic, natural antibacterial substances has been in great demand (Davies, 1994; Enne, More, Stephens and Hall, 2001; Poole, 2001).

During past few years, there has been increasing interest in isolating and recovering important Components from agricultural Waste to add value addition to the residues. The emergence of Sustainable development by encouraging Controlled exploitation of agricultural waste for economic gains will help to add value addition to the coffee husk.

2. Materials and methods

2.1. Chemicals

Methanol, ethyl acetate, ethanol, ethyl ether, sulphuric acid, acetic acid, chloroform, Folin and Ciocalteu's reagent, sodium carbonate were from Ranbaxy, India; gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), potassium iodide, potassium hydroxide, 3-carotene, linolenic acid, Tween 40 were from Sigma Chemicals, USA; potassium ferric cyanide, trichloroacetic acid, sodium phosphate, ammonium molybdate were from BDH, India; nutrient agar was from HiMedia, Mumbai. Green tea polyphenols (GTP) and rosemary were from Flavors and Essence Ltd. Mysore, India. Refined sunflower oil (Sun pure) was procured from the local market.

2.2. Instrumentation

Visible spectra measurements were done using Specord 50 UV-Vis spectrophotometer with 1.0-cm silica quartz matched cell.

2.3. Plant material

Well ripened coffee fruit was collected from coffee plantation of Western Ghats in India. The fruits were squeezed separated into pulp, seed and peel. Only the peel was used as husk.

2.4. Sample preparation

Coffee peel was washed with water to remove the adhering pulp and dried in hot air oven at 60°C to remove moisture and the dried husk was ground into fine powder. The material that passed through a 40-mesh sieve was used for further studies.

2.5. Preparation of extract

Coffee husk (50 g) was transferred to a 1000 ml round bottom flask 200 ml of 70% (v/v) methanol was added and refluxed for 45 min at 80°C on a water bath. The extract was filtered through filter paper and the residue was reextracted under the same conditions for five times.

The filtrate was combined and evaporated in a rotary evaporator below 60°C. The procedure was repeated using different solvents like hexane, ethyl acetate, methanol and water. The extract obtained after evaporation of organic solvents was analyzed for the content of polyphenols and the results are reported in Table 1.

Table 1: Total phenolic content in CHE

Extracts	Polyphenols (%)	Polyphenols (%) after extraction with ethyl acetate
Hexane	1.13	3.54
Methanol	6.31	10.12
Ethyl acetate	4.21	4.21
Water	3.10	6.25
Methanol(70% w/v)	7.30	13.12

The extracts collected as above were reextracted 5 times with ethyl acetate, the combined ethyl acetate portion was evaporated in rotary evaporator below 60°C, The extract after evaporation of ethyl acetate was used as natural antioxidant, Percentage of polyphenols enriched after the above process is presented in table 1.

2.6. Determination of total phenolic content

The phenolic components were determined according to the procedure described by Negi, Jayaprakash and Jena (2003), Twenty milligrams of extract were dissolved in 50 ml of 50% (v/v) methanol and filtered through Whatman no 44 filter paper. An aliquot of 0.4 ml of sample was taken in a 25-ml standard flask and mixed with 1 ml of Folin and Ciocalteu's reagent (1:10 diluted with water) and allowed to react for 5 min, 1 ml of 10% sodium carbonate was added and allowed to stand for 90 min at room temperature, and the absorbance of the reaction mixture was read at 725 nm. Standard graph was prepared using gallic acid. The content of total polyphenols in the extract was calculated from the standard graph and expressed as percent concentration in the extract,

2.7. Quenching of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical by CITE

The method described by Kitts, Wijewickreme and Hu (2000) was used to assess the DPPH radical scavenging capacity of CHE.

A 0.1 mM DPPH solution in ethanol was mixed with 20, 40, 60, 80, 100 and 120 µg/ml of CHE and vortexed thoroughly. The mixtures were allowed to stand at ambient temperature (22±3 oC) for 30 min. The absorbance was measured at 519 nm using a spectrophotometer. The scavenging percentage was calculated according to the following equation.

$$\text{Scavenging, \%} = \frac{(\text{Abs Control} - \text{Abs Sample}) \times 100}{\text{Abs Control}}$$

2.8. Antioxidant activity assay using sunflower oil

Refined sunflower oil, free of additives, was used as the substrate for oxidation studies. Sunflower oil samples containing 1000 ppm of CHE and natural antioxidants (GTP and rosemary) were separately prepared. Each 250 ml prepared oil sample was placed in a 500 ml brown airtight glass bottle. Synthetic antioxidants (BHT) were mixed with the oil for comparative study at their permitted legal limits of 100 ppm (Duh and Yen, 1997). Control samples of sunflower oil without antioxidant were also placed under identical conditions. All oil samples in each treatment were prepared in triplicate and were stored at room temperature (22 ± 3 oC) for 80 days. Oil samples from each treatment were withdrawn periodically to assess the antioxidant activity of CHE.

2.8.1. Antioxidant activity measurement

The rate of oxidation was followed by periodic determination of POV of stored oils. Each sample (1 g) was put into 250-ml Erlenmeyer flasks and dissolved in a mixture of 25 ml of acetic acid/chloroform (3:2, w/v). Saturated solution of potassium iodide (1 ml) was added and the flask was placed in a dark chamber for 5 min, after which 75 ml distilled water was added. The liberated iodine was titrated with a sodium thiosulfate solution (0.01 M) in the presence of starch indicator (AOAC, 1990).

2.8.2. Free fatty acid content

Free fatty acid content was determined at regular intervals in stored sunflower oil. Ten grams of each sample were weighed into an Erlenmeyer flask and 50 ml of ethanol/ethyl ether (1:1 v/v) mixture were added. This suspension was titrated with 0.1M potassium hydroxide (KOH), using 1 ml of phenolphthalein indicator, until faint permanent pink color persisting for 30 sec was obtained. (AOAC, 1990).

2.9. Antioxidant assay using β-carotene-linoleate model system

The antioxidant activity of CHE was evaluated using β-carotene-linoleate model system as described by Jayaprakasha, Sing and Sakariah (2001). β-carotene (0.2 mg) in 0.2 ml of chloroform, 20 mg of linolenic acid and 200 mg of Tween-40 (polyoxyethylene sorbitan monopalmitate) were mixed. Chloroform was removed at 40°C under vacuum and the resulting mixture was diluted with 10 ml of Water and was mixed well. To this 40 ml of oxygenated water was added. Four milliliter aliquots of the emulsion were pipetted into different test tubes containing 0.2 ml CHE (100, 200, 300, 400 µg) and BHT (100, 200, 300, 400 µg) in ethanol. BHT was used for the comparative study. A control containing 0.2 ml of ethanol and 4 ml of the above emulsion was prepared. The tubes were placed in 50 oC in water bath and the absorbance at 470 nm was taken at zero time (t = 0 min). Measurement of absorbance was continued till the color of β-carotene disappeared in the control tubes (t = 120 min) at an interval of 15 min. A mixture prepared as above without β-carotene served as blank. All determinations were carried out in triplicates. The antioxidant activity (AA) of the extract was evaluated based on the extent of bleaching of the β-carotene using the formula.

$$AA = \frac{100 [1 - (A_o - A_t)]}{[A_o^o - A_o^t]}$$

Where, A_o and A_{0o} are the absorbance values measured at zero time of the incubation for test sample and control, respectively. A_t and A_{0t} are the absorbance measured in test sample and control after incubation for 120 min.

2.10. Reducing power of CHE

The reducing power of CHE was determined according to the method of Oyaizu (1986). Different amounts of extract (0.24, 0.60, 1.20, 1.80, 2.40 mg/ml) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 ml, 1%). The mixture was incubated at 50 oC for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture and centrifuged for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1 %), and the absorbance was measured at 700 nm. Absorbance of the reaction mixture increased with the increase in reducing power.

2.11. Antibacterial activity

Bacterial cultures of *Escherichia coli* (Gram -ve), *Staphylococcus aureus* (Gram +ve), *Salmonella typhi* (Gram -ve) and *Salmonella enterica* were obtained from Defense-Food Research Laboratory, Mysore, India. The cultures were grown in nutrient agar (HiMedia, Mumbai India), at 37°C. Each bacterial strain was transferred from stored slants at 4 -5°C to 10 ml broth and cultivate overnight at 37°C. A preculture was prepared by transferring 1 ml of this culture to 9 ml broth and cultivate for 48 h. The cells were harvested by centrifugation (1200g, 5 min), Washed and suspended in saline. The CHE was tested against the bacteria by the method of Negi, Jayaprakah, Rao and Sakariah (1999). To flasks containing 20 ml melted cool agar, different concentrations of test material in Tween 40 were added. Equivalent amounts of Tween 40 were used as Controls. One hundred microliter (about 10³ cfu/ml) of each bacterium to be tested Was inoculated into the flasks under aseptic conditions. The media were then poured into sterilized Petri plates in duplicate and incubated at 37°C for 20-24 h. The colonies, after the incubation period were counted and expressed as colony forming units per ml of culture (cfu/ml). The minimum inhibitory concentration (MIC) was reported as the lowest concentration of the CHE required for inhibiting the complete growth of the bacterium being tested.

3. Results and discussion

The % yields of CHE in hexane, ethyl acetate, methanol, water and aqueous methanol (70% w/v) solvents were 2.5, 4.5, 8.5, 5.6 and 17.9, respectively. The total polyphenols contents of CHE as determined by Folin-Ciocalteu method are reported as gallic acid equivalents (Table 1). Amongst the solvents used aqueous 70% (v/v) methanol gave the highest yield of polyphenolic extract. The order of the efficiency of the solvents examined is aqueous methanol (70% v/v) > methanol > ethylacetate > water > hexane. The total phenolics determined are not absolute measurements but are based on their chemical reducing capacity relative to gallic acid. In the present study the responses of the CHE may arise from the variety and/or quantity of phenolics found in extracts of coffee husk.

3.1. Free radical scavenging activity of CHE

The scavenging activity of CHE phenolics compared to gallic acid for DPPH radical is shown in Table 2. The results indicate a concentration-dependent scavenging activity of the DPPH radical.

Further it was found that the scavenging activity of CHE was found to be maximum at 120 g/ml and it was 81.82%.

Table 2: Percent radical-scavenging activity on DPPH by CHE and gallic acid

Extract concentration ug/ml	CHE (% scavenging)	Gallic acid (% scavenging)
20	39.10 ± 0.84	94.13 ± 0.42
40	55.41 ± 1.01	94.71 ± 0.25
60	73.32 ± 1.14	94.34 ± 0.34
80	77.10 ± 0.95	94.83 ± 0.25
100	80.01 ± 1.00	94.71 ± 0.27
120	81.82 ± 1.09	94.82 ± 0.41

Mean values Standard Deviations (n=3)

3.2. Antioxidant activity of CHE in refined sunflower oil

Oxidation of lipids is undesirable as it affects the flavor, aroma, nutritional quality and even the texture of a product. The chemicals produced by the oxidation of lipids are responsible for rancid flavor and aroma. BHT, GTP and rosemary are commonly used antioxidants. BHT is a synthetic while GTP and rosemary are natural antioxidants. We have determined the antioxidant property of CHE and compared the values with the three reference antioxidants. The data presented in Table 3 and 4 manifest that CHE has antioxidant properties comparable to that of GTP and rosemary. However, CHE was found to be not as powerful as that of BHT in its antioxidant activity. The decrease in FFA and POV clearly indicates that auto oxidation of Sunflower oil was greatly inhibited by the CHE. This study revealed that the extract can be used as a natural antioxidant at a little higher concentration compared to natural antioxidants like GTP and rosemary studied.

3.3. Antioxidant activity using β -carotene-linoleate model system

The data regarding the antioxidant activity using β -carotene-linoleate model system of CHE at 100, 200, 300 and 400 μg concentrations are presented in Table 5. Addition of CHE and BHT prevents the bleaching of β -carotene to different degrees. β -carotene depending upon their concentration undergoes rapid discoloration in the absence of an antioxidant. This is due to the coupled oxidation of β -carotene and linolenic acid, which generates free radicals. The linolenic free radicals formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups attacks the highly unsaturated β -carotene molecules. As a result, β -carotene gets oxidized and broken down in part, subsequently the system loses its chromophore and characteristic orange color; this can be monitored spectrophotometrically. In the present study, the CHE was found to hinder bleaching of β -carotene by neutralizing the linoleate free radical and other free radicals formed in the system. CHE showed 91 % antioxidant activity when used at 400 μg concentrations.

Table 3: Effect of synthetic antioxidant BHT (100 ppm), natural antioxidants GTP (1000 ppm) and rosemary (1000 ppm) and CHE (1000 ppm) on peroxide value (mmol oxygen / kg) during storage of sunflower oil.

Storage period days	Control	BHT	GTP	Rosemary	Coffee husk
1	7.60 ± 1.10	6.98 ± 1.07	7.23 ± 1.14	7.22 ± 1.07	7.23 ± 1.17
5	15.76 ± 1.35	9.77 ± 1.17	13.91 ± 1.58	9.96 ± 1.45	13.01 ± 0.98
10	30.89 ± 1.88	12.62 ± 1.82	20.21 ± 1.18	21.19 ± 1.04	20.40 ± 1.40
15	33.50 ± 2.41	13.10 ± 1.18	27.60 ± 1.78	30.88 ± 2.57	30.36 ± 1.47
20	44.12 ± 1.92	15.77 ± 1.67	36.85 ± 1.47	37.24 ± 1.48	39.06 ± 1.52
25	49.72 ± 2.15	16.85 ± 1.87	41.15 ± 2.17	46.60 ± 2.40	47.10 ± 1.91
30	62.44 ± 2.31	17.29 ± 1.92	45.57 ± 2.47	52.78 ± 1.82	51.03 ± 2.48
35	70.34 ± 2.14	18.59 ± 1.97	51.33 ± 2.38	65.95 ± 2.48	58.19 ± 2.27
40	73.79 ± 1.85	21.15 ± 1.98	53.18 ± 2.12	72.82 ± 2.17	62.61 ± 2.52
45	78.17 ± 2.41	25.71 ± 1.67	57.36 ± 2.18	77.30 ± 2.30	70.54 ± 2.54
50	88.55 ± 1.98	31.18 ± 1.18	68.88 ± 2.17	82.51 ± 1.68	85.06 ± 2.48
60	114.61 ± 1.84	41.87 ± 1.95	80.76 ± 2.08	87.76 ± 2.32	104.33 ± 1.68
70	144.12 ± 1.58	46.37 ± 1.10	92.30 ± 2.14	99.33 ± 1.97	108.15 ± 1.55
80	161.58 ± 1.68	55.00 ± 1.05	112.07 ± 2.11	109.09 ± 2.06	130.47 ± 1.53

Mean values ± Standard Deviations (n=3)

Table 4: Effect of synthetic antioxidant BHT (100 ppm), natural antioxidants GTP (1000 ppm) and rosemary (1000 ppm) and CHE (1000 ppm) on FFA (mg KOH/g sample) during storage of sunflower oil.

Storage period days	Control	BHT	GTP	Rosemary	Coffee husk
1	0.24 ± 0.02	0.25 ± 0.04	0.24 ± 0.03	0.24 ± 0.05	0.25 ± 0.05
5	0.27 ± 0.03	0.28 ± 0.03	0.28 ± 0.02	0.26 ± 0.04	0.28 ± 0.01
10	0.36 ± 0.01	0.33 ± 0.03	0.31 ± 0.04	0.32 ± 0.02	0.34 ± 0.02
15	0.41 ± 0.02	0.40 ± 0.02	0.38 ± 0.03	0.40 ± 0.05	0.41 ± 0.05
20	0.47 ± 0.05	0.46 ± 0.05	0.42 ± 0.01	0.47 ± 0.04	0.48 ± 0.05
25	0.52 ± 0.01	0.49 ± 0.04	0.47 ± 0.02	0.54 ± 0.04	0.55 ± 0.07
30	0.56 ± 0.04	0.54 ± 0.06	0.50 ± 0.05	0.59 ± 0.06	0.59 ± 0.05
35	0.62 ± 0.06	0.60 ± 0.08	0.53 ± 0.07	0.64 ± 0.10	0.66 ± 0.07
40	0.71 ± 0.07	0.64 ± 0.05	0.58 ± 0.07	0.71 ± 0.07	0.73 ± 0.09
45	0.81 ± 0.06	0.68 ± 0.09	0.62 ± 0.09	0.77 ± 0.09	0.80 ± 0.08
50	1.02 ± 0.11	0.70 ± 0.08	0.69 ± 0.11	0.80 ± 0.08	0.88 ± 0.12
60	1.04 ± 0.09	0.76 ± 0.12	0.81 ± 0.10	0.88 ± 0.11	0.95 ± 0.11
70	1.23 ± 0.08	0.83 ± 0.09	0.91 ± 0.12	0.96 ± 0.12	0.99 ± 0.12
80	1.27 ± 0.11	0.87 ± 0.09	1.02 ± 0.11	1.06 ± 0.12	1.01 ± 0.09

Mean values ± Standard Deviations (n=3)

Table 5: Antioxidant activity of extract from CHE and BHA by β -carotene-linoleate model system

Sample	Concentration (μg)	Antioxidant activity (%)
CHE	100	58.21 ± 0.84
	200	79.30 ± 0.41
	300	88.10 ± 0.41
	400	91.03 ± 0.97
BHT	100	89.61 ± 0.09
	200	95.14 ± 0.14
	300	96.13 ± 0.11
	400	96.03 ± 0.05

Mean values ± Standard Deviations (n=3)

3.4. Reducing power

Table 6 shows the reducing capacity of CHE compared to BHT. For this we investigated the $\text{Fe}^{3+}/\text{Fe}^{2+}$ transformation in the presence of CHE, using the method of Oyaizu (1986). Earlier authors (Pin-Der-Dhu, 1998; Pin-Der-Duh, Pin-Chan-Du and Gow-Chin Yen 1999; Tanaka, Kuie, Nagashima and Taguchi, 1998) have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductones (Pin-Der-Duh, 1998), which have been shown to exert antioxidant action by breaking the free radical chain by

donating a hydrogen atom (Gordon, 1990). Reductones also react with certain precursors of peroxide, thus preventing peroxide formation. The data (Table 6) on the reducing power of CHE suggests that it is likely to contribute significantly towards the observed antioxidant effect. Like the antioxidant activity, the reducing power of CHE increased with increasing amount of Sample. However, the reducing power of BHT was relatively more pronounced than that of CHE.

Table 5 : Reducing power of CHE at different concentration

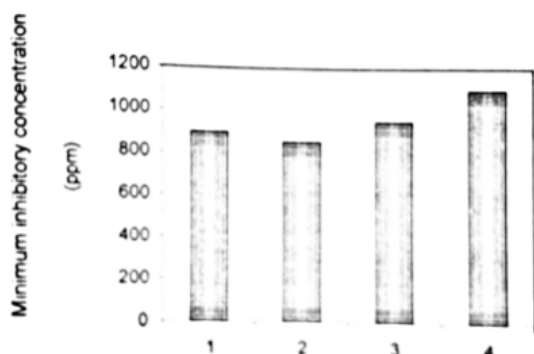
Amount of CHE (µg/ml)	Absorbance at 700nm	
	CHE	BHT
00	0.01 ± 0.01	0.01 ± 0.00
50	0.08 ± 0.01	0.18 ± 0.04
100	0.17 ± 0.03	0.38 ± 0.01
150	0.28 ± 0.00	0.58 ± 0.01
200	0.37 ± 0.04	0.62 ± 0.01
250	0.46 ± 0.02	0.73 ± 0.04
300	0.55 ± 0.01	0.85 ± 0.01

Mean values ± Standard Deviations (n=3)

3.5. Antimicrobial activity

The effect of CHE on the growth of four different bacteria is presented in Figure 1. CHE inhibited growth of bacteria to variable extent, depending on the bacterium in question. CHE was more effective against *S. aureus* and at 800 ppm complete inhibition of bacterial growth was observed and this was followed by *E. coli*, *S. typhi* and *S. enterica* which required 850, 950, 1100 ppm, respectively for complete growth inhibition.

Fig 5 : The effect of CHE on the growth of *E. coli* (1), *S. aureus* (2), *S. typhi* (3) and *S. enterica* (4).



4. Conclusion

First-ever studies on coffee husk have been carried out to recover potential polyphenols which have demonstrated antioxidant and antimicrobial properties. The study has been carried out to develop natural and cost-effective material from agro-industrial residue. The extraction procedure adopted has indicated high antioxidant and antibacterial activity. Further studies are in progress individual phenolic regarding the characterization of underlying compounds and to elucidate the mechanism bioactive properties and existence of possible synergism, if any, among these compounds. In summary, agriculture is a predominant sector of World's economy and has reached a plateau through the use of external input-intensive technologies. These technologies helped to increase demand of feed, food and functional foods for teeming billions, but, such technologies are resource degrading and environmental polluting. In such a situation, agricultural production cannot sustain for an everlasting period. Thus, for sustainable agricultural development there is an urgent need for an effective management of agricultural waste. Reuse, regeneration and recovery are the three components of recycling-one of the important curative approaches in environmental management.

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