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Short Communication

Total phenol and antimicrobial properties of green coconut husk extract

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Abstract

Ethanol (70%) hot water (45±2°C) and a combination of 70% ethanol and hot water (60:40 mL) were used as solvents to produce an extract from green coconut husk. The antioxidant properties of the extract were evaluated by estimating the total phenolic content (TPC) using the Folin-Ciocalteu reagent, while its antimicrobial effect on selected bacteria was investigated using the agar well diffusion method. The highest TPC was observed in the ethanolic coconut husk extract (ECHE) with 313.44±0.26 mg GAE/g, followed by a mixture of ethanol and hot water coconut husk extract (MEHWCHE) with 300.35±0.25 mg GAE/g, while the lowest concentration was found in the hot water coconut husk extract (HWCHE) around 269.76±0.25 mg GAE/g. The antimicrobial activity of these extracts against *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus* showed that ECHE had higher inhibition zones for all species compared to MEHWCHE and HWCHE. Coconut husk, a widely available byproduct of *Cocos nucifera*, contains significant levels of phenolic compounds with potent antioxidant and antimicrobial properties. Optimized unconventional extraction methods can efficiently isolate these bioactive compounds, providing a sustainable and eco-friendly solution for valorizing coconut husk waste while addressing pollution issues. The aim of the current study is to evaluate the potential to extract beneficial plant-based compounds from coconut husks using ethanol and hot water, which could be utilized as dietary polyphenols or natural food preservatives.

Keywords: Agar well diffusion, Antimicrobial activity, Coconut husk extract, Folin-Ciocalteu reagent, Total phenolic content

Highlights

- Coconut husk extract is a rich source of phenolic compounds.
- The antibacterial properties of coconut husk extract, combined with its antioxidant potential, suggest it is a viable natural alternative for antimicrobial and antioxidant applications.

Plants are the source of various classes of phytochemicals such as phenolic compounds, also known as polyphenols, which are the secondary metabolites of plants involved in protection against ultraviolet radiation or pathogenic aggression (Genwali *et al*., 2013). Polyphenols are classified into major groups like flavonoids, tannins, phenolic acids, coumarins, hydroxyl cinnamic acids and lignans as well as quinones, curcuminoids, and stilbenes (Maqsood *et al*., 2013). Dietary polyphenols have gained much attention due to their potent antioxidant properties and possible role in preventing various diseases associated with oxidative stress. *Cocos nucifera* is an important fruit in tropical countries (Rodiah *et al*., 2018), where Asia accounts for 84.2% of the world's coconut production in 2018 (FAOSTAT, 2020). The morphology of coconut fruit (a drupe) contains three layers of exocarp, mesocarp, and endocarp from the fruit wall. Usually, a mature coconut consists of coconut water (25%), and white meat (28%), which is covered by a robust protective shell (12%) and a thick husk (35%) (Van Dam *et al*., 2004). The mesocarp becomes hard and fibrous due to extensive cross-linking between phenolics, lignin and polysaccharides. The composition of coconut husk contains 80% lingo cellulosic materials, which are mainly used to produce low-tech fiber mattresses and biomass fertilizers. They are even directly discarded and burned, resulting in waste of resources and pollution (Quinones *et al*., 2020; Schena *et al*., 2020). However, it is also the main contributor to the nation's pollution problem as solid waste (60%) in the husk,

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which involves an annual production of approximately 3.18 million tones (Adkins *et al*., 2006). Few studies have found that coconut husk contains a high concentration of antioxidant and antimicrobial phenolic compounds such as tannin, hydroxybenzoic acid, ferulic acid, syringic acid, coumaric acid, and vanillic acid (Chakraborty and Mitra, 2008; Valadez *et al*., 2016; Olatunde *et al*., 2019). These compounds can be efficiently extracted using optimized extraction methods and will exhibit significant antibacterial activity against selective bacteria. Mazaya *et al.* (2020) reported that coconut shell extract exhibited notable antimicrobial properties, particularly against *Acetobacter aceti*. The most potent extract was obtained from a dry, aged coconut shell after 3 hours of extraction at 28°C. This extract contained key phytochemicals, including tannins, saponins, and steroids, and had a total phenolic content of 18.67 mg per 100 g. Uy *et al*. (2019) investigated the antimicrobial and antioxidant properties of *Cocos nucifera* roots. Their findings revealed that the root extract exhibited antimicrobial activity against *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*. Phytochemical analysis identified the presence of tannins, saponins, alkaloids, steroids, glycosides, and flavonoids, all of which are known for their antioxidant properties. There are various conventional and unconventional extraction techniques for determining phenolic compounds from plant materials. Traditional methods such as solid-liquid, liquid-liquid, and maceration extraction are labor-intensive and inconsistent. To address these issues, new unconventional techniques have been developed, including pressurized liquid extraction, subcritical water extraction, supercritical fluid extraction, and microwave-assisted extraction. These unconventional methods provide improved properties like automation, enhanced selectivity, higher efficiency, and reduced solvent use. Coconut husk, a widely available byproduct, contains significant levels of antioxidant and antimicrobial phenolic compounds. Optimized extraction methods can efficiently isolate these bioactive compounds, providing a sustainable solution for valorizing coconut husk waste and addressing pollution. The study aims to evaluate the potential to extract beneficial plant-based compounds from coconut husks using ethanol, which could be utilized as dietary polyphenols or natural food preservatives.

The green coconut was collected from the local market of New Garia, Kolkata, West Bengal. After collecting the raw material, it is transported to the processing hall of the Faculty of Fishery Sciences, Kolkata. The husk was separated from the coconut shell manually. The separated husk was cleaned with potable water to remove dirt and dust. After drying in a hot air oven (45±2°C) the coconut husk was cut into small pieces, and the separation of powder was done manually to prepare the extract. The dried coconut husk powder is stored in a zipper bag at room temperature for further analysis. For the present study, ethanol is used as a solvent for extracting phenolic compounds due to its classification as a generally recognized safe solvent, making it particularly suitable for preparing extracts with pharmacological or nutraceutical interest (Santos *et al.*, 2012). For solvent extraction methods, three different types of solvent ethanol (70%), hot water (45±2°C), and a mixture of 70% ethanol and hot water (60:40 mL) were used. For each 10 g of the sample, 100 mL of solvent was mixed and frequently stirred with a glass rod. After keeping the mixture for the whole night, the sample vortexed at high speed for 5 minutes and centrifuged at 5000 rpm for 10 minutes. After that, the sample was filtered through a Whatman no.1 filter paper. The prepared extracts were considered as stock extracts and labelled as ethanolic coconut husk extract (ECHE), hot water coconut husk extract (HWCHE), a mixture of ethanol and hot water coconut husk extract (MEHWCHE), filled in the glass bottles and stored at 4±2°C until further analysis.

The total phenolic content of the coconut husk extract was determined by the Folin-Ciocalteu method described by Lin and Tang (2007), and the results were expressed as grams of gallic acid equivalents (mg GAE)/g of dried coconut husk. Coconut husk extract (0.1 mL) was mixed with 2.8 mL of distilled water and 0.1 mL of 50% Folin-Ciocalteu reagent. To this, 2 mL of 7.5% sodium carbonate was added. It was mixed well and incubated in the dark at room temperature for 60 minutes. The absorbance was taken at 765 nm in a spectrophotometer. All experiments were carried out in triplicate, and the respective solvents were used as blanks. Gallic acid was chosen as a standard using a seven-point standard curve (25-300 μg/mL). The antibacterial activity of different solvent extracts of coconut husk was evaluated by the agar well diffusion method (Verma *et al*., 2012) for *Escherichia coli* (EHEC strain EDL 933), *Bacillus cereus* (MTCC 1306), and *Staphylococcus aureus* (ATCC- BAA- 976). *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus* are strategically selected for testing the antimicrobial activity of coconut husk extracts due to their public health relevance, diverse

Table 1. Characteristics of the different solvent extracts of coconut husk

Total phenolics (mg GAE/gram dry skin)			
Properties	**ECHE**	**HWCHE**	**MEHWCHE**
Colour	Dark brown	Slightly yellow to brown	Slightly dark brown
pH	4.19±0.43	3.86±0.39	4.41±0.47
Total phenolic content (TPC)	313.44^{c}±0.26 mg GAE/g	269.76^{a}±0.25 mg GAE/g	300.35^{b}±0.23 mg GAE/g

Values are presented as the mean ± standard error (n = 3), p< 0.05). ECHE = Ethanolic coconut husk extract, HWCHE = Hot water coconut husk extract and MEHWCHE = Mixture of ethanol and hot water (60:40) coconut husk extract

biological characteristics, and suitability for standardized testing. These bacteria represent both Gram-positive and Gram-negative groups, encompassing strains known for their resistance to conventional antibiotics. This makes them ideal candidates for evaluating coconut husk extracts as potential alternative antimicrobial agents. The bacteria were grown by inoculating a loopful of the culture in the nutrient broth (5 mL) and incubated at 37°C for 8 to 12 h. The fresh bacterial culture was uniformly spread over the Muller Hinton Agar plates using sterile cotton swabs and left to air dry. With the help of a 6 mm cup borer a well was created in the plates for agar well diffusion. Subsequently, 500 µL of each extract was carefully applied into the well to ensure it dried without overflowing. The plates were then placed in an incubator at 37°C for 24 hours, after which the zone of inhibition around the well was measured for antimicrobial activity. The results obtained were then subjected to statistical analysis using One-Way Analysis of Variance (ANOVA) and the Duncan test to determine the significant differences ($p<0.05$) between the means of parameters analyzed. Table 1 summarises the basic properties of the coconut husk extracts prepared with different solvents. ECHE had a darker colour than HWCHE and MHWCHE, which could be attributed to the concentration and type of solvent used. The pH values of ECHE, HWCHE and MEHCHE were 4.19±0.43, 3.86±0.39, 4.41±0.47, respectively.

The result of TPC from coconut husk extract was calculated from the regression equation of the standard plot of gallic acid (Y= 0.0034x+0.0983, R2=0.999) (Fig. 1). The TPC in ECHE, HWCHE and MEHCHE were found to be 313.44±0.26 mg GAE/g, 269.76±0.25 mg GAE/g and 300.35±0.25 mg GAE/g respectively. The extraction of bioactive compounds from coconut husk is the first step in using phytochemicals to make dietary supplements, nutraceuticals, food ingredients, food preservatives and pharmaceuticals (Dai and Mumper, 2010). The solvent extraction procedure is a very efficient and helpful method to prepare plant

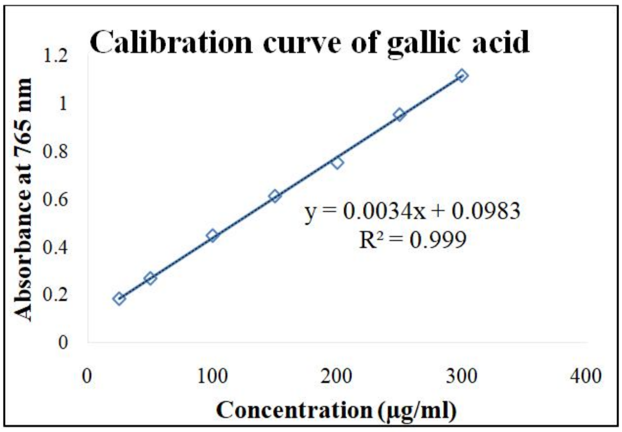

Fig. 1. Calibration curve of gallic acid

extract. Numerous factors, including the solvent type and polarity, sample-to-solvent ratio, extraction time and temperature, as well as the chemical composition and physical properties of the samples, have a significant impact on the yield of chemical extraction (Dai and Mumper, 2010). Ethanol is an appropriate solvent for extracting polyphenols from various plant materials and is considered safe for human consumption (Shi *et al*., 2005). Our study revealed that ethanol exhibited the highest overall phenol extraction efficiency, followed by a combination of ethanol and hot water, and finally hot water alone. Increasing the solvent concentration from 60% to 80% resulted in a significant increase in TPC, while using 100% solvent can decrease the TPC yield (Buamard and Benjakul, 2015; Olatunde *et al*., 2019). Dey *et al*. (2003) found that mature coconut dried mesocarp husk had around 13 mg GAE/g of total phenolic content. Phenolic compounds in the mesocarp decrease with age, as they become associated with the cell wall or used in lignin synthesis (Chakraborty and Mitra, 2008). Additionally, TPC levels may vary due to differences in coconut husk biology, location, and species. Buamard and Benjakul (2015) suggested that extracting active phenolic compounds from coconut husk was less efficient when the ethanol concentration was greater than 60%. As a result, the polarity of the coconut husk powder extraction solvent had a significant effect on TPC and extraction yield (Sultana *et al*., 2009)

Based on the TPC analysis, it was found that high polar solvents such as ethanol and a mixture of hot water and ethanol were more effective in recovering polyphenols from coconut husk. On the other hand, hot water alone was less efficient. Therefore, the polyphenols present in coconut husk in this study could be classified as highly polar.

Table 2. Diameter of inhibition zone (mm) of different solvent coconut husk extract against some pathogenic bacteria using agar well diffusion

Bacterial sp.	ECHE	HWCHE	MEHWCHE
Escherichia coli	$13.66^{ab}\pm0.88$ mm	$10.83^{a}\pm0.60$ mm	$12.50^{ab}\pm0.28$ mm
Staphylococcus aureus	$14.66^{b}\pm0.72$ mm	$10.66^{a}\pm0.33$ mm	$13.00^{b}\pm0.10$ mm
Bacillus cereus	$15.5^{ab}\pm1.25$ mm	$10.50^{a}\pm0.50$ mm	$12.16^{ab}\pm0.71$ mm

Values are presented as the mean ± standard error (n = 3), p< 0.05). ECHE = Ethanolic coconut husk extract, HWCHE = Hot water coconut husk extract and MEHWCHE = Mixture of ethanol and hot water (60:40) coconut husk extract

The antimicrobial activity of various solvent extracts of coconut husk against selected microorganisms varies. The ethanolic extract from coconut husk showed the maximum zone of inhibition against *Bacillus cereus* was 15.5±1.25 mm, 14.66±0.72 mm against methicillin-resistant *Staphylococcus aureus*, and 13.66±0.88 mm against *E. Coli* (Fig. 2). The hot water extract of coconut husk exhibited antimicrobial activity with zones of inhibition measuring 10.83±0.60 mm against *E. coli*, 10.66±0.33 mm for methicillin-resistant *S. aureus*, and 10.50±0.50 mm against *Bacillus cereus* (Table 2). Furthermore, the ethanol and hot water combination exhibited the highest antimicrobial effectiveness, producing inhibition zones of 13.00±00 mm against methicillin-resistant *S. aureus* (Fig. 3), 12.50±0.28 mm for *E. coli*, and 12.16±0.71 mm for *B. cereus* (Fig. 4) respectively.

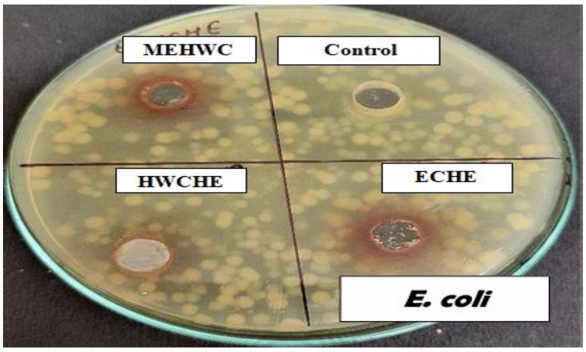

Fig. 2. Antimicrobial properties of coconut husk extracts on *E. coli*

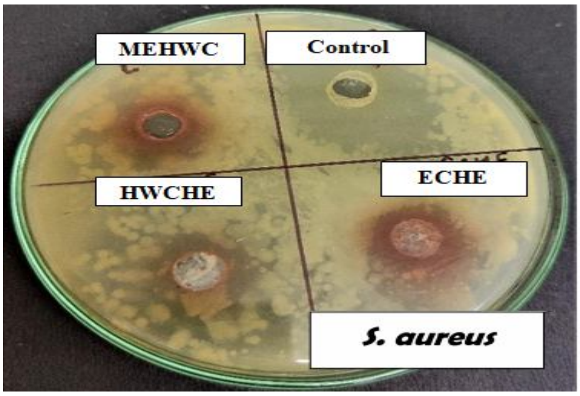

Fig. 3. Antimicrobial properties of coconut husk extracts on *S. aureus*

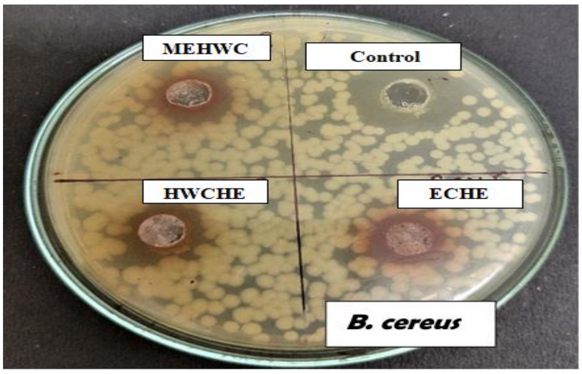

Fig. 4. Antimicrobial properties of coconut husk extracts on *B. cereus*

Essential oils and extracts have long been used as natural therapies. Plant extracts show promise as sources of novel antimicrobial compounds, particularly against bacterial pathogens. The beneficial medicinal effects of plant materials, including antibacterial activity, are typically attributed to the combination of secondary metabolites present in the plant (Cyriac *et al*., 2013). The primary phenolic compounds identified in coconut husk include gallic acid, 4-hydroxybenzoic acid, vanillic acid, ferulic acid, syringic acid, vanillin, catechin, kaempferol, epicatechin, and rutin (Valadez *et al*., 2016), as well as caffeoylquinic and dicaffeoylquinic acids (Chakraborty and Mitra, 2008). Esquenazi *et al*. (2002) highlighted that catechins, B-type procyanidins, and other phenolic compounds are key contributors to the antimicrobial activity exhibited against various bacteria. The hydrophobic nature of phenolic compounds may contribute to their antimicrobial activity by allowing them to partition the lipids of the bacterial cell membrane and mitochondria, causing disruption and making the cell structures more permeable (Parekh *et al*., 2006). Extensive leakage from bacterial cells and the exit of critical molecules and ions will result in death (Jose *et al*., 2014). Several extrinsic and intrinsic parameters determine the inhibition produced by plant extracts against a specific organism. The diffusivity of plant extract in agar medium may also influence the zone of inhibition (Cyriac *et al*., 2013). Phytochemical screening of *C. nucifera* conducted by Alviano *et al*. (2008) has reported that this plant material is rich in alkaloids, flavonoids, catechin, epicatechin, and condensed tannins, which confer its potent antimicrobial properties. Extracts from coconut husks, when treated with various solvents, demonstrate notable inhibitory effects against prevalent pathogens, suggesting the existence of highly potent antimicrobial substances.

The present research indicates that coconut husk extract offers a rich source of plant phytochemicals with promising antioxidant and antibacterial properties. The choice of solvents used in the extraction process influences both the phenolic content and antibacterial effectiveness of coconut husk. This study provides valuable insight for selecting appropriate solvents when extracting bioactive compounds from coconut husk.

Conflict of interest: All authors declared that there is no conflict of interest.

Author's contribution: PS: Conceptualization, methodology, formal analysis, original draft writing; KCD: Formal analysis, investigation, visualization and supervision; SR: Methodology and editing; KS: Conceptualization, visualization and editing; RM: Methodology, and formal analysis.

Data availability statement: The data mentioned in the article will be available with the corresponding author.

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