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Phytochemical analysis and antimicrobial activity of coconut husk extract on various solvents

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Abstract

Coconut is known as the "wonder food" and is regarded as perfect diet because it contains almost all essential nutrients needed by the human body. Coconut husk is a by-product of the coconut that known contains phytochemical compounds and antimicrobial activities. This study aims to determine the phytochemical characteristics and the antimicrobial activity of coconut husk extracts. Completely Randomized Design was used in this study and the effect of coconut husk and the different solvent type were investigated. These are selected Five different solvents, they are polar solvent and non-polar i.e. Acetone, Ethanol, Methanol, Chloroform and Petroleum ether. The phytochemicals characteristic and antimicrobial activity of coconut husk extract were determined. The results of this study indicated that coconut husk extract containing Carbohydrates, Reducing sugar, Sugar, Proteins, Tannins, Saponin, Flavonoids, Steroids. and terpenoids. The coconut husk extracts showed antimicrobial activity against Streptococcus cocci and E. coli and the microbes isolated from soil. The coconut husk extracted with Acetone show the highest antimicrobial activity. The highest antimicrobial activity against Streptococcus cocci bacteria with inhibition zone diameter of 10 mm, and the coconut husk extract with Ethanol show the lowest inhibition antimicrobial activity these are 04 mm against E. coli. The extract was observed to be more effective against Streptococcus cocci. Presence of tannins and other phenolic compound ds may responsible for antimicrobial activity.

Keywords: Coconut husk; Phytochemical; Antimicrobial

1. Introduction

Coconut is a drupe borne by the coconut palm (*Cocos nucifera*), a member of the monocotyledonous family Palmae. It is known as the "wonder food" and is regarded as perfect diet because it contains almost all essential nutrients needed by the human bod. *Cocos nucifera* (family Aceraceae) commonly known as coconut, is considered as an important fruit crop in tropical countries. It is a versatile plant with a variety of uses. Every part of it is useful to mankind for several purposes including food, drinks, fibers, building materials and chemicals finding their way into a huge range of modern-day products [1]. Coconut has a wide family which consists of 217 genera and 2,500 species. Cocos nucifera belongs to the order Arecales and it is the sole species of the genus cocos which belongs to the subfamily cocoideae, which includes 27 genera and 600 species [2]. Coconut palms are grown in more than 80 countries of the world, with a total production of 61 million tons per year [3]. India is the third largest coconut producing country, having an area of about 1.78 million hectares under the crop. In India, the four south Indian states namely Kerala, Tamil Nadu, Karnataka and Andhra Pradesh account for around 90% of the coconut production in the country [4]. Coconut is a very versatile and indispensable fruit for most people under the tropical belt. It is a complete food is rich in calories, vitamins, and minerals. It is nourishing, strengthening and fattening food. It has high oil content. The protein is of high quality and contains all amino acids essential for the growth and maintenance of the body. It is rich in K, Na, Mg and S. The energy value of the dried coconut is 662 calories per 100 g [5]. The nutrient content of nuts varies by species, but in general they provide

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rich sources of vegetable protein, monosaturated and polyunsaturated fatty acids, dietary fiber, vitamins E & K, folate, magnesium, copper, selenium and potassium. Nuts are also naturally low in saturated fatty acids and sodium [6]. Coconut is planted for different purpose (nutritional and medicinal) and that is the reason why it's called the fruit of life [7], [8]. *C. nucifera* produced different products which include coconut water, coconut husk, copra, coconut oil, raw kernel, coconut cake and coconut milk. It is a unique source of different natural products in the development of drugs and industrial products that is effective against fungi, bacteria, viruses, parasites and dermatophytes [9]. Modern medical science is now confirming the medicinal qualities which are used for the treatment of heart, liver and kidney disorders. Based on the knowledge of the traditional herbs used for the treatment for local application, coconut husk can be used as a topical antimicrobial. As preliminary investigation of the use of coconut husk, the antimicrobial activity can be evaluated [10], [11], [12]. When fibrous coconut husk is used for cleaning the teeth, it is understandable that the fibers will mechanically remove the colonized microorganisms from the tooth surface. As the earlier studies have revealed some antimicrobial properties, it is possible that an additional beneficial effect of this material, that is, the antimicrobial effect against oral pathogens, may also be contributing to improve the oral health [13].

As one of the highest coconut-producing regions in Indonesia, North Sulawesi contributes to almost 10% of the amount of Indonesian coconut. However, from the high amount of coconut production, most of the existing coconut husk is still a waste and its benefits are still not widely enjoyed by the people in North Sulawesi. One form of industry that is seen as having the potential to be developed and suitable for small to medium scale is the processing of husk to be used as fiber and coir ash and to develop it to be some more values such as carpets, ropes, etc. In addition to its good market prospects, husk is the largest heavy component (38-44%) of the coconut fruit, compared to other components such as shell (21-28%) and coconut water (29-35%) [14]. This husk can be processed into coconut fiber and coconut powder (cocopeat). Coconut fiber can be used, among others, as raw material for industrial carpets, upholstery, vehicle dashboards, mattresses, and hardboards, while coconut peat can be used as a medium for horticultural crops. Other products include coconut pot, and coconut fiber board. These materials are raw materials in the mat industry, pots, dry compost, and soon [15]. In order to have coconut fiber and cocopeat, a coconut husk processing machine is needed [14]. Although the potential is great, the coconut husk processing business is relatively unreachable by small-scale farmer groups. This is due to the lack of husk processing equipment to make fiber and coconut peat. Existing tools are often too expensive and difficult for small-scale farmers to afford. The potential for increasing added value in the coconut agroindustry is immense [16]. Non-food goods made from processed coconut include activated carbon, shell powder, fibre, charcoal, handicrafts, furniture, and roofs. Coconut husk is composed of organic and mineral elements, namely: pectin and hemicellulose, lignin and cellulose, potassium, calcium, magnesium, nitrogen, and protein. Comparison of the above components depending on the age of the coconut husk, lignin in coconut husk fiber ranges from 40% - 50%. Coir fibre has high buoyancy, is resistant to Bacteria, saltwater and is cheap, while its weakness is that it cannot be twisted properly and is classified as a stiff fibre. The quality of coconut fibre is determined by color, percentage of dirt, moisture content, and the proportion between the weight of long and short fibres. Derived products from coconut husk in the form of coconut fibre, coir dust. Processing of coconut husk into coir fibre and coconut coir dust can be done by biological and mechanical methods [17]. The biological fibre is carried out by utilizing the role of microorganisms to soften the coconut husk, the coconut husk is soaked in water, so it is more popularly known as immersion fibre.

In the lot coconut producer areas, coconut husk often caused the disposal of coconut husks in the field. However, if left continuously, it can even result in the fields being unkempt and dirty. On the other hand, when coconut husk is burned, the smoke generated is often very stinging and disturbing the environment. One of the efforts to reduce the negative impact is to utilize coconut husk waste into a valuable product. Whereas in some places, this coconut fibre has been used as a composite material that can improve its mechanical properties, which is cheap and environmentally friendly [17]. Unfortunately, its utilization in Indonesia is still very limited compared to its capacity. About 60% of domestic agricultural waste is from coconut husk [18] The coconut fruit wall consists of three layers which are the outer epicarp (skin), mesocarp (fibrous husk and coir) and endocarp (hard shell). Previous research reported that husk fibre from the shell and coir possessed antibacterial, antiviral, antifungal, antileishmanial and antioxidant activities [19]. Phenolic compounds and bioactive substances in plants are components that contribute to biological activities in plant cells such as antioxidant and antimicrobial activities. Previous studies mentioned the content of phytochemical compounds in coconut husk such as tannins, saponins, steroids, lignin, and saturated and unsaturated fatty acids [20], [21]. pentosans, cellulose, catechin and epicatechin [19]. These compounds contribute to biological activities such as antioxidant, antimicrobial, antiviral, antileishmanial and cytotoxic properties. Catechin possesses cellular growth inhibitory property, thus contributes to anticancer, antimicrobial, antimutagenic and anti-inflammatory properties. Previous studies have shown that coconut husk have antimicrobial properties.

Utilization of the bioactive components contained in coconut husk will be easier when the extraction process was carried out. Extraction is the process of separating a substance from its mixture using a solvent. The result of extraction process is influenced by several factors such as the polarity of the solvent, the ratio of the material and solvent, and the

length of the extraction time. The selection of the appropriate solvent will make the extraction process efficient [22]. So far, the effect of solvent polarity on the content of phytochemical compounds in coconut husk extracts is unknown and related to this research is still limited. Therefore, it is necessary to conduct research to determine the appropriate type of solvent to obtain the highest phytochemical compounds and antimicrobial activity in coconut husk extract. This study aims to determine the Analysis of phytochemical compounds and antimicrobial activity in coconut husk extract in different solvent types.

2. Material and methods

2.1. Collection of Material

The Mature (7-8 months) coconut husk was collected from local coconut growers and local Market of Chhatrapati Sambhajinagar. Outer husk of the fruit was decorticated, the husk fibers were washed with distilled water to remove dirt, cut into small size of 1x1 cm and cabinet air-dried for 21 days. The dried husk fiber was then blended using household electric blender, for the preparation of powder [Figure 1].

2.2. Selection of Solvents

The Non-Polar Solvents and Polar Solvents were taken for Coconut husk extraction. Non-Polar Solvents are Chloroform and Petroleum ether. The polar solvents are of 2 types namely Polar-Aprotic and Polar-Protic Solvents are Acetone, Methanol and Ethanol [23].

2.3. Extraction

About 100 g powder of the Coconut husk was extracted using 250 ml of solvent (Acetone, Methanol, Ethanol, Chloroform and Petroleum Ether) at 100°C using Soxhlet extractor. The extract was concentrated on water bath. Residue obtained was transferred into glass vial and stored at temperature 4 °C.

2.4. Qualitative Phytochemical Determination [24]

Obtained extract was evaluated for various Phyto-constituents by various Phyto-chemical qualitative tests.

2.4.1. Test for Carbohydrates

- **Molisch's Test: -** 1ml of sample extract was put into a test tube add few drops of alpha naphthol and 1ml of H2SO4. If a Violet ring is detected, the sample is positive for Carbohydrate compounds.

2.4.2. Test for Reducing Sugar

- **Fehling's Test: -** When Fehling - A and Fehling - B solution mix at 1:1 ratio and add few drops of test sample to test tube then place the test tube in a water bath at 60ºC. Brick red colour precipitate indicate presence of Reducing sugar.
- **Benedict's Test: -**1ml of test sample must be mixed with 2ml of Benedict's Reagents and heated in a water bath of boiling water for 3 to 5 minutes. The development of a brick-red coloured precipitate of cuprous oxide confirms the presence of reducing sugars.

2.4.3. Test for sugars

- **Test for Pentose sugar:** -1ml of sample extract was put into a test tube add 2 drops of HCL and crystals of phloroglucinol. If a Red color absent indicate absence of pentose sugar.
- **Test for Hexose sugar: -** Heat selwinoffs reagent and 1.5ml sample extract in water bath for 1-2 min. If a Red colour absent indicate absence of Hexose sugar.

2.4.4. Test for Protein

Biuret Test: - 1-2 ml of sample extract was put into a test tube add 1-2 ml of Biuret reagent. shake well and allow the mixtures to stand for 5 minutes. If a Solution turns from blue to deep purple, it indicates positive for Proteins.

2.4.5. Test for Flavonoids

1 ml of sample extract was put into a test tube and 1 ml of 10% lead acetate was added. If a yellow or orange color is detected, the sample is positive for flavonoid compounds.

2.4.6. Test for Steroids

2 mL of sample extract was put into a test tube, then 2 ml of chloroform ($CHCl_3$) and 2 mL of concentrated sulfuric acid (H_2SO_4) were added. If a red-brown ring is detected between the layers of the solution, the sample is positive for steroid compounds.

2.4.7. Test for Terpenoids

A total of 2 ml of sample extract was put into a test tube and then 2 ml Acetate $(CH_3CO)_2$ and 2–3 drops of concentrated sulfuric acid (H_2SO_4) were added. If a dark red color is detected, the sample was positive contains terpenoid compounds.

2.4.8. Test for Saponin

5 ml of sample extract was put into a test tube and 5 ml of Aquadest was added and then heated. If the foam detected on the surface, the sample is positive for saponin compounds.

2.4.9. Test for Tannins

2 ml of sample extract was put into a test tube, then 2 ml of Aquadest and 2-3 drops of 5% FeCl3 were added. If a green precipitate is detected, the sample is positive for tannin compounds.

2.5. Test for Alkaloid

- **Mayer's Test: -** 2 ml of sample extract was prepared in a test tube then added 5 drops of Mayer reagent. If a white or yellow lumpy sediment is formed the sample is positive for alkaloid compounds.

Table 1 Phytochemical Test of Coconut Husk Extract with Different Solvent

Sr. No.	Name of Solvent Extract	Carbohydrate	Reducing Sugar		Sugar		Protein
		Molisch's Test	Fehling's Test	Benedict's Test:	Pentose sugar	Hexose sugar	Biuret Test
1	Acetone	+	+	+	-	+	+
2	Methanol	+	+	-	+	-	-
3	Ethanol	+	-	+	+	+	+
4	Chloroform	+	+	+	-	+	-
5	Petroleum Ether	-	-	-	-	-	-

Positive Test: +Negative Test: -

Table 2 Qualitative Phytochemical Characteristics Test of Coconut Husk Extract with Different Solvent

Sr. No	Name of Solvent Extract	Flavonoids Test	Steroids Test	Terpenoids Test	Saponin Test	Tannins Test	Alkaloids Mayer's Test
1	Acetone	+	+	+	+	+	+
2	Methanol	+	-	-	-	+	+
3	Ethanol	+	+	+	+	+	+
4	Chloroform	+	-	-	-	+	-
5	Petroleum Ether	-	+	-	-	+	+

Positive Test: + Negative Test: -

2.6. Isolation and identification of test organisms

The strains of *Staphylococcus cocci* and *E. coli* were isolated from soil source by using Nutrient Agar media. After the growth of Bacteria on Petri Plates, the Morphological observations of colonies were external features, Colony color, Shape of colony, Surface of Colony, Colony Pattern, growth rate, Opticity of colony, Margin of Colony, Elevation of colony

& microscopic characteristics of shape, size and spore colour of Bacteria. Macroscopic & microscopic features of Bacteria were helpful inaccurate identification of Bacteria. The identification of bacteria was done by using various research papers, monographs & other literature such as, Practical Atlas for Bacterial Identification [25], Biochemical Test for Identification of Medical Bacteria [26].

2.7. Composition of media used in isolation of Bacteria:

2.7.1. Nutrient Agar Media

Sodium chloride (NaCl) 10.0g, Peptone 10.0g, Yeast Extract 1.0g, Agar-Agar 15.0g, p^H was maintained 7.5, Distilled water 1000ml.

2.7.2. Tested Organisms

The bacterial strains were belonging to the gram positive (*Streptococcus cocci*) and gram negative (*E. coli*) were taken for tests. All the above bacterial strains were prepared in Nutrient Broth were maintained 40ºC. Active culture inoculums for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes containing nutrient broth and incubated for 24 h at 370 ºC.

2.7.3. Antimicrobial Activity

The anti-bacterial screening was carried out using agar diffusion method [27] with slight modifications. Freshly prepared inoculum was swabbed all over the surface of the agar plate using sterile cotton swab. Five wells of 5 mm diameter were bored in the medium with the help of sterile cork-borer having 5 mm diameter and were labeled properly and 5ml concentrations of Coconut husk extract and same volume of positive and negative control were filled in the wells with the help of micropipette. 5 ml of Acetone, Methanol and Ethanol was used as a positive control and the solvent control distilled water. After incubating the plates at 37°C for 24 hours, the zone of inhibition was measured using a scale. The mean and standard deviation of triplicates of various concentrations of plant extract were calculated and compared with solvent.

Table 3 Antimicrobial Activity of coconut husk extract on tested organisms

Sr. No.	Name of Solvent	Inhibition Zone (mm) Against *Staphylococcus cocci*	Inhibition Zone (mm) Against *E. coli.*
1	Acetone	10	06
2	Methanol	06	07
3	Ethanol	08	04

3. Results and discussion

3.1. Qualitative Phytochemical Test

The results of the phytochemical analyses Test carried out on *Cocos nucifera* husk revealed the presence and the absence of some secondary plant metabolites. From the result it was detected that 70% of the phytochemical test were present in husk fibre, Phytochemical analysis showed the plant to contain Carbohydrate, Sugar, Reducing Sugar, Protein, Alkaloids, Flavonoids, Saponins, Tannins, Steroid and Terpenoid [Figure 2, 3]. The Phytochemical test shows that the Carbohydrates, Reducing Sugar, Sugar, Protein and Alkaloids are dominantly present in Acetone and Ethanol Extract of Coconut husk. The petroleum Ether are showing the lowest Prescence of all compound except the Alkaloids (Table 1). The qualitative phytochemical test showed that tannin and flavonoid compounds were detected in Acetone, Methanol, Ethanol, Chloroforms extract for coconut husk, and tannin also detected in Petroleum ether extracts of coconut husk. Steroid compounds were detected in Coconut husk extracts of Acetone, Ethanol and Petroleum ether. Saponin compounds were detected in Acetone and Ethanol extract of Coconut husk, similarly the Terpenoid compounds were detected in the Acetone and Ethanol extract of coconut husk. In the coconut husk extract, tannin and flavonoid compounds were detected and the color intensity stronger than the other coconut husk extract. This is because the level of maturity is an important factor that affects the composition and amount of phytochemical compounds in plants [28]. Acetone and Ethanol solvents confirmed to be more effective in extracting phytochemical compounds than Methanol, Chloroform and Petroleum Ether solvents. This is indicated that the phytochemical compounds in coconut husk are

dominated by polar compounds, so the effective solvents for extracting phytochemical compounds are polar solvents such as Acetone and Ethanol. (Table 2).

3.2. Antimicrobial Activity

According to [29], tannin compounds have acidic properties and have strong activity at weak acid p^H stated that flavonoids are slightly acidic. Based on this literature, it is suspected that most of the phytochemical compounds have acidic properties and this is the reason for the lowest in p^H that occurred in the coconut husk Acetone extract. The antimicrobial activity of coconut husk extract against *Streptococcus cocci* was shows the highest than to other *E. coli* [Table 3 & Figure 4]. It is suspected that this is because *Streptococcus cocci* is a microbe that is quite strong, has strong fermentative properties, biochemical stability, and ability to reproduce well in a propagation medium, more tolerant of acidic environments and has several important enzymes in the decomposition of organic compounds.

The diameter inhibition zone of coconut husk extract against *Streptococcus cocci* was greater than the inhibition zone of coconut coir extract against *E. coli*. It is suspected due to *E. coli* which is included in the group of gram-negative bacteria whose cell walls contain 10–20% peptidoglycan. Outside the cell wall is a capsule. The function of capsules is to defend the cell from antibiotic produced by other microbes [30]. The presence of a peptidoglycan layer and capsule on *Streptococcus cocci* caused this bacterium to be more resistant to antimicrobial agent from coconut husk extract. Antimicrobial activity of coconut husk with acetone extract was highest antimicrobial activity against *Streptococcus cocci* with an inhibition zone diameter of 10 mm. The diameter of the inhibition zone of Acetone extract was the highest among other extracts, followed by Methanol and Ethanol extract. The lowest zone of inhibition is observed in Ethanol Coconut husk extract it is 04 mm against *E. coli*. This is due to the effect of the pH of the extract and the phytochemical content of the coconut husk in extract.

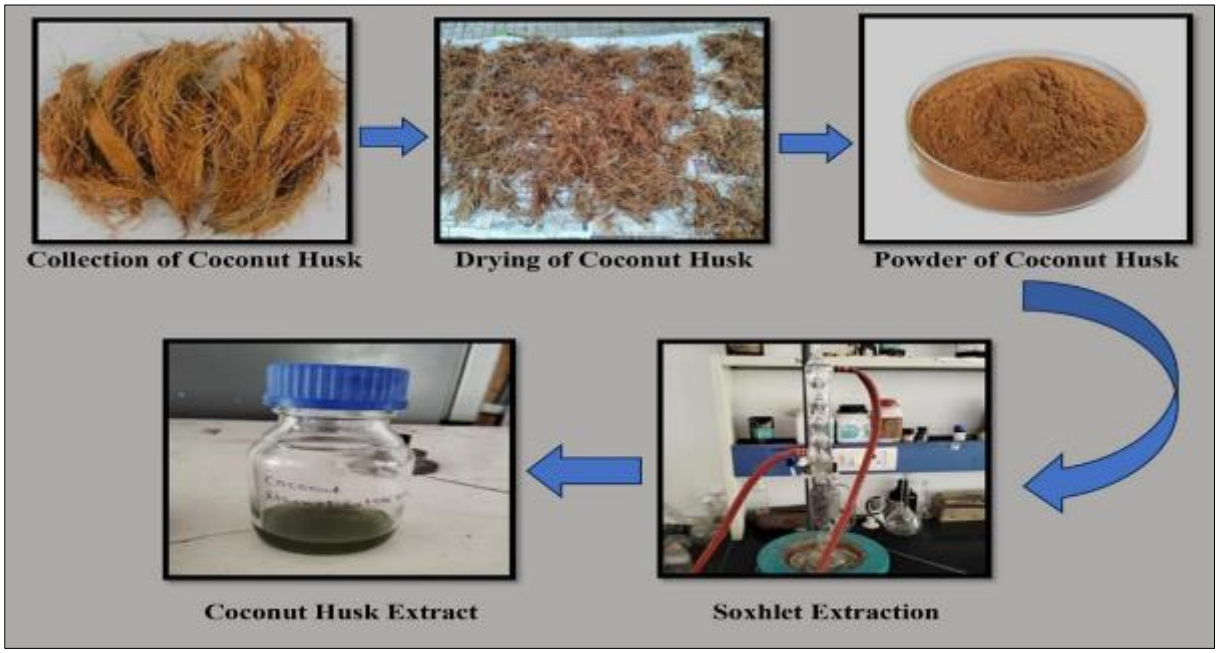

Figure 1 Preparation of coconut husk extract using different solvent

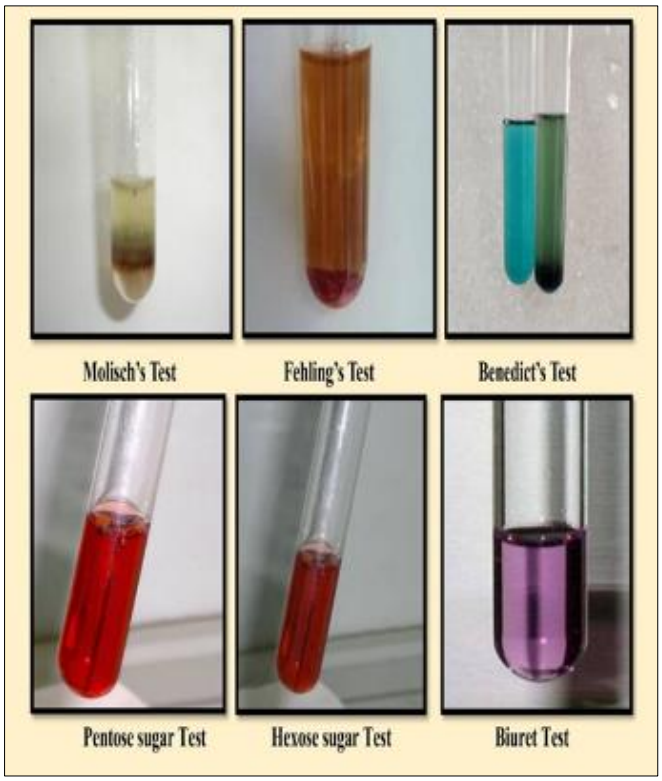

Figure 2 Qualitative phytochemical test of coconut husk

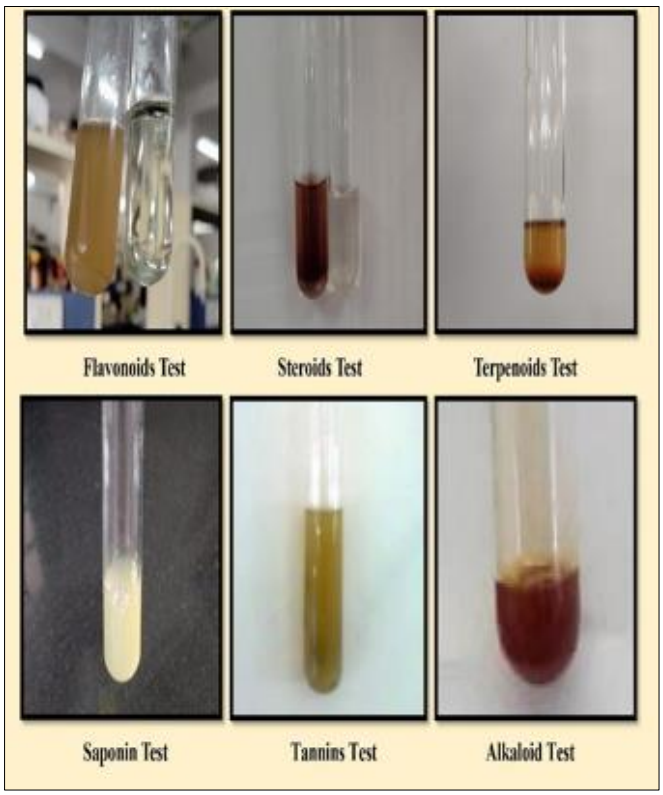

Figure 3 Qualitative phytochemical test of coconut husk

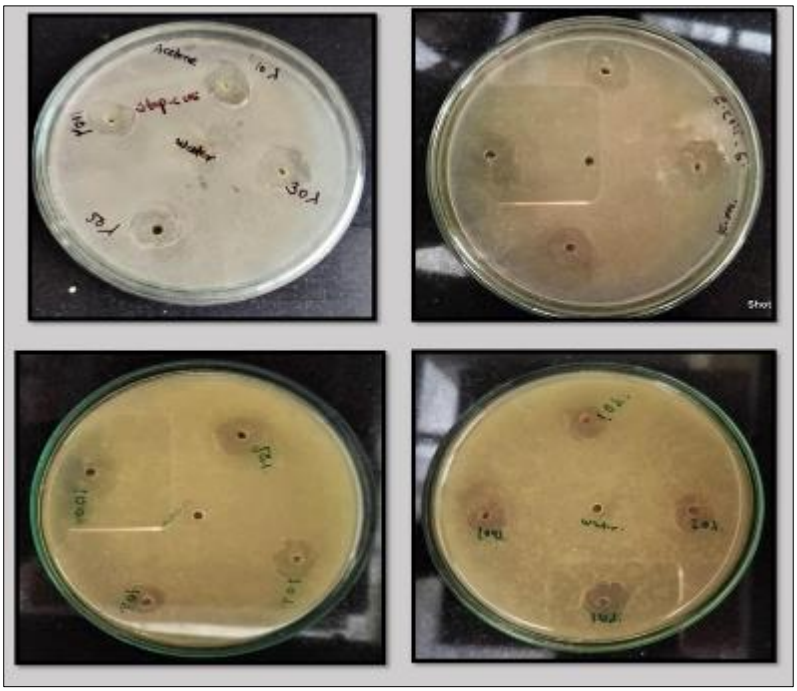

Figure 4 Antimicrobial activity of coconut husk extract *Streptococcus cocci* and *E. coli* bacteria

4. Conclusion

The results of this study indicated that the coconut husk extract containing Carbohydrates Reducing Sugar, Sugar, Protein, Alkaloids, Tannins, and flavonoids, Steroids, Terpenoids and Saponin. The coconut *husk* extracts showed antimicrobial activity against *Streptococcus cocci* and *E. coli* the microbes isolated from soil. The coconut husk extracted with Acetone show the highest antimicrobial activity against all microbial tested. This extract contains a total flavonoid and tannin. Coconut husk has a significant inhibitory action against common pathogens, indicating the presence of highly effective antimicrobial compounds. This study may be used as guidance in the selection of suitable solvents in the extraction of secondary metabolites compounds from coconut husk. Further detailed research still needs to be done to develop antibacterial or antimicrobial agents from coconut husk.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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