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Analysis of the Bactericidal and Phytochemical Features of Multiple Fruit Wastes

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Abstract
The potential characteristics of herbal remedies have made them promising since the dawn of humanity. Because they contain nutritious and nutrient-rich substances, fruits are consumed around the world both fresh and processed, yet their peels, skins, seeds, and other parts are rarely used or even discarded. If they are not properly disposed of, this contributes to a significant pollution issue. To gain better understanding of their phytochemical and antibacterial capabilities, the wastes of Punica granatum, Citrus limetta, Musa paradisiaca, Ananas comosus, Zea mays, and Annona squamosa were investigated. These could prove to be an innovative source of antimicrobials and be used to combat harmful microorganisms. E. coli, Bacillus subtilis, and Pseudomonas aeruginosa were used as test organisms for the antibacterial characteristics (NCIM 2065, 2196, and 5029, respectively). These fruit scraps showed good potency, and it is speculated that they could work well as a bactericidal agent.

Key words: *Antimicrobial activity, gentamicin, inhibition, phytochemical.*

I. Introduction

India is an agricultural nation, and nature has endowed it with a wide variety of fruits, vegetables, and crops. Many fruits and fruit wastes have long been researched for their potential as medicines and treatments. To research the qualities of these fruits and the fruit waste, Punica granatum, Citrus limetta, Musa paradisiaca, Ananas comosus, Zea mays, and Annona squamosa were chosen.

The Punicaceae family includes the pomegranate, Punica granatum, sometimes known as anar or dalim. ellagic tannins, ellagic acid, and gallic acid are among the polyphenols found in high concentrations in the peels of Punica granatum. 1. These plants' potential therapeutic uses as analgesics, antimicrobials, antipyretics, and cancer preventatives might all be established. 2, 3.

One of the most significant tropical fruits on the global market is the banana, which belongs to the Musaceae family. Musa peels are produced in large amounts as waste, accounting for 40% of the weight of fresh bananas. Vitamins A, C, gallocatechin, dopamine, E, B6, sitosterol, malic, succinic, and palmitic acids, as well as minerals including magnesium, phosphorus, potassium, and iron, are all present in musa peels. These elements in Musa peel are what give it its anti-microbial properties. 4. Sweet lime is often referred to as sweet lemon or Citrus limetta. The Rutaceae, or Rue family, contains the genus Citrus. In addition to vitamins, minerals, dietary fiber, essential oils, and carotenoids, citrus limetta is a rich source of phenolic compounds. Before being discarded, the peel of C. limetta serves as a vital bio-resource for beneficial bioactive substances because to its high vitamin C and fiber content. 5. The majority of bioactive substances are also utilized by the food and cosmetic industries. Additionally, it has been utilized as a mutagenic, antiviral, anti-viral, anti-hepatotoxic, and anti-diabetic drug. 6. Pineapple, or Ananas comosus, is a member of the Bromeliaceae family.7, 8. In many local cultures, it is widely utilized as a medicinal plant, and the bromelain in pineapple is thought to be responsible for its healing powers. A basic extract called bromelain includes a number of proteinases in addition to other substances. Numerous fibrinolytic, antiedematous, antithrombotic, and anti-inflammatory effects are present. The use of bromelain as a phytotherapeutic agent dates back to its discovery as a chemical. 9. The fibers found in the crown leaves of Ananas comosus are multicellular lignocellulose, which includes cellulose (75%), hemicellulose (16%), and lignin (9%).

The primary cereal crop in the globe is corn, or Zea mays L., which is a member of the Poaceae family. When combined with other cereal flours, maize flour offers nutritive and effective qualities as food components. 10. The husk of Zea mays contains significant amounts of minerals, particularly calcium, potassium, aluminum, silicon, and phosphorus.

The Annona squamosa, also known as the custard apple, sitaphal, or sugar apple, is an abundant source of calcium, phosphorus, iron, carbohydrates, and protein. Annona squamosa peels have tannins and astringent qualities that are utilized to create herbal supplements. Alkaloids and acetogenin, two natural substances that lower the risk of cancer and renal failure, are abundant in the fruit. It works against cancer cells while having no negative effects on healthy cells. Additionally discovered to have anti-helminthic qualities are antioxidants like asimicin and bullatacin. 11. Solid wastes frequently have a high nutritional content and can be used as animal feed, especially when they result from procedures like peeling and coring 12.

The growing number of diseases linked to microbes worries everyone. To many antibacterial substances created up to this point, germs have grown significantly more resistant. The advancements made in antibiotic research over the last fifty years have been fast undone by the appearance of antibiotic-resistant microbes 13. It has a lengthy history of microbial agent resistance, which has changed how drugs are developed. Fruit peels and other fruit debris can be utilized as natural antibiotics. These days, a lot of research is done on this to learn more about its biochemical characteristics. They can be used in place of other agents to combat various bacteria. In this regard, the antibacterial activity and phytochemical test analysis of Punica granatum, Citrus limetta, and Musa paradisiaca peel, Ananas comosus crown leaves, Zea mays husk, and Annona squamosa fruit peel extracts in methanol, ethanol, chloroform, and hexane were studied.

II. Materials and Methods

Collection of fruit waste (peels): The plant material was procured from a local market in Saharanpur and included the peels of Punica granatum (pomegranate), Citrus limetta (sweet lime), Musa paradisiaca (banana), Ananas comosus (pineapple), Annona squamosa (custard apple), and Zea mays (maize).

Chemicals and reagents: Methanol, ethanol, chloroform, hexane and DMSO of Merck (Germany) were used. Gentamicin, nutrientagar, nutrient broth and other chemicals were purchased in Hi- media.

Micro-organisms and culture: The test micro-organisms like *Escherichia coli* (NCIM 2065), *Bacillus subtilis* (NCIM 2196) and *Pseudomonas aeruginosa* (NCIM 5029) were obtained from NCIM Pune and Chandigarh. These isolated subcultures are nutrient agar and nutrient broth for further studies.

Preparation of the extracts: The collected fruit wastes were thoroughly washed with double distilled water. The peels were cut into small slices and dried at 35°C for 2-6 hours. The dried materials were grinded in a mixer and made into powder. The powder was stored in airtight plastic bags at -20°C.

The Soxhlet apparatus was assembled using different polar and non-polar solvents like ethanol, methanol, chloroform and n- hexane extract. Powdered peels of fruit plant material (20 g) was soxhleted in 200 ml of solvents for 6-18 h. All extracts were concentrated over an evaporator until a powder or semi solid extract sample was collected and stored in 4°C for further analysis.

Determination of antimicrobial activity of different fruit peel extracts: Antibacterial activities of the extracts were studied byagar well diffusion method [14]. Test cultures of the bacteria wereprepared by transferring a loop full of bacteria from nutrient agar slants into nutrient broth and incubated at 37°C. Stock cultures of the test organisms were prepared by swabbing sterile nutrient agar plates with 24 h old bacterial broth. Wells were punched with a sterile cork borer (8 mm internal diameter) and 100 µl of the extract was added to each well. Controls were maintained with respective solvents. Gentamicin (50 µg/ml) was used as a standard antibiotic for Gram positive and Gram negative bacteria. After incubation at 37°C for 24 h, diameters of the inhibitory zones weremeasured to the nearest millimetre scale.

The antibacterial activity of methanol, ethanol, chloroform and n-hexane extracts of *Punica granatum, Citrus limetta, Musa paradisiaca, Ananas comosus, Zea mays* and *Annona reticulata* peel at concentrations of 50, 60, 70 and 80 mg/ml were tested against Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram positive bacteria (*Bacillus subtilis*), and gentamicin was used as a positive control.

Preparation of inoculums: The antimicrobial activities of crude extracts obtained by different solvents were screened against the bacteria *Escherichia coli* (NCIM 2065), *Pseudomonas aeruginosa* (NCIM 5029) and *Bacillus subtilis* (NCIM 2196). Stock cultures were maintained at 4°C on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cellsfrom the stock cultures to test tubes of nutrient agar for bacteriaincubated at 24 hours at 37ºC. The assay was performed by welldiffusion method.

Phytochemistry analysis: Qualitative chemical analysis of fruit waste plant extract was done. The methanol, ethanol, chloroform and n-hexane and the peel powder were subjected to qualitative test like carbohydrates, reducing sugars, deoxy sugars, protein, sterols, glycosides, phenolic compounds, tannins, saponins and flavonoids test.

III. Results and Discussion

Antibacterial activity of fruit waste extracts

Punica granatum (pomegranate) peel: The agar well diffusion method was used to test the antibacterial potency of Punica granatum peel extract (Fig. 1A-B). Methanolic and ethanolic extracts had the strongest antibacterial effects.In comparison to Bacillus subtilis and Pseudomonas aeruginosa, Escherichia coli was the most susceptible to the methanolic extract (80 mg/ml). In comparison to other extracts like ethanol, chloroform, and hexane, the extract showed the highest inhibitory activity of methanol (16 mm) against E. coli and P. aeruginosa. When compared to P. aeruginosa and B. subtilis, the largest zone of inhibition by an ethanol extract at an 80 mg/ml concentration was seen in E. coli (Fig. 1A). E. coli, B. subtilis, and P. aeruginosa all displayed a comparable zone of suppression when exposed to 80 mg/ml of chloroform extract (Fig. 1B). Effective zone of inhibition in E. coli and B. subtilis for n-hexane extracts was at 80 mg/ml concentration. Additionally, it has been discovered that the pomegranate peel ethanol extract possesses inhibitory zones that range from 9.6 0.5 to 18.6 1.1 mm against a variety of Gram negative and Gram positive microorganisms. 18.Citrus limetta (sweet lime) peel extracts (Fig. 1C-D) showed the strongest antibacterial activity against E. coli, P. aeruginosa, and B. subtilis at an 80 mg/ml concentration. Similar zones of inhibition were shown against E. coli, P. aeruginosa, and B. subtilis in methanolic extract at an 80 mg/ml concentration (Fig. 1C), however in ethanolic extract, the same zones of inhibition were observed between E. coli and P. aeruginosa in comparison to Bacillus subtilis. In comparison to other extracts such as ethanol (8 mm), methanol (8 mm), and hexane (8 mm), the extract showed the greatest inhibitory efficacy in chloroform (11 mm) against P. aeruginosa. When compared to E. coli and B. subtilis, P. aeruginosa showed the most efficient inhibition in the n-hexane extracts at 80 mg/ml (Fig. 1D).

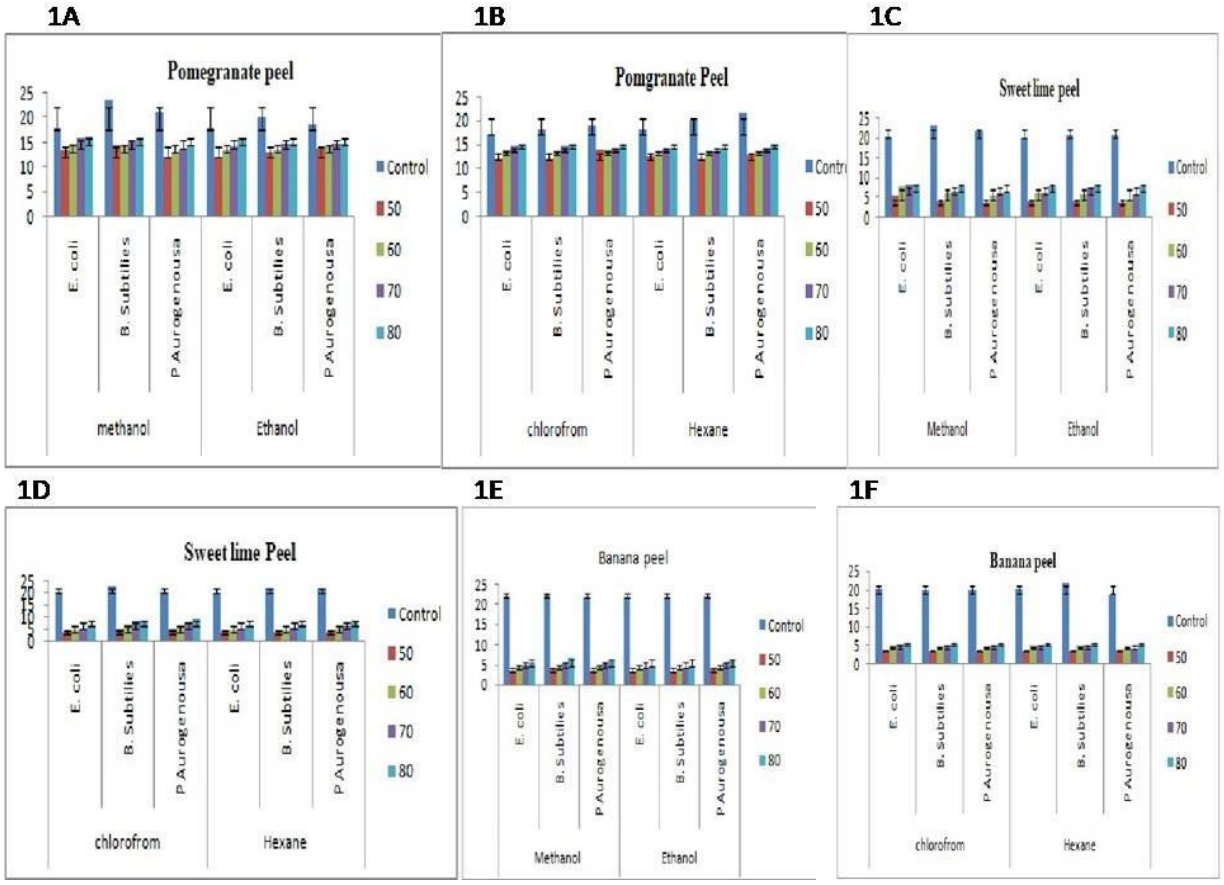

Figure 1. Structure of pomegranate (A-B), sweet lime (C-D) and banana (E-F) methanol, ethanol (polar solvent), chloroform and hexane (non-polar solvent) peel extracts.

The drug resistance with the comparative study for methanol, ethanol, chloroform and n-hexane extracts of *Citrus limetta* peels show the antibacterial potency of four different extracts ranges from 50 to 80 mg/ml. Unnisa *et al.* [15] reported that the ethanolic extracts of fruit waste exhibited antibacterial activity. The ethanolic extracts of pomegranate also exhibited highest antibacterial activity among all five fruits used with diameter of inhibition zone values between 20 and 26 mm. Maximum inhibitory effect was recorded for *S. aureus* 26 mm,

followed by *E.coli* 25 mm, *P. aeruginosa* 22 mm and *K. pneumoniae* 20 mm. Similar findingswere also reported by some other workers. The aqueous extract of peel also showed lower (0.25 mg/g) ascorbic acid content as compared to alcoholic extract (0.25 mg/g) [21, 22] .The data revealed that the alcoholic extract showed higher inhibitory effect againstall bacterial strain than their aqueous extract.

Musa paradisiaca (banana) peel: The antibacterial potency of *Musa paradisiaca* (Fig. 1 E-F) methanolic extract of 80 mg/ml was most effective in *B. subtilis* (inhibition zone ±7 mm) as compared to other extracts like ethanol (±6 mm), chloroform (±6 mm) andhexane (±6 mm) of *E. coli, B. subtilis* and *P. aeruginosa* (Fig. 1E).The ethanolic extract (80 mg/ml) showed the maximum zone ofinhibition in *P. aeruginosa*. The chloroform extract (80 mg/ml)exhibited the highest zone of inhibition in *E. coli* as compared to *B. subtilis* and *P. aeruginosa* (Fig. 1F). The n-hexane extract (80mg/ml) had the highest zone of inhibition against *B. subtilis* compared to *E. coli* and *P. aeruginosa*. The drug resistance with methanol, ethanol, chloroform and n-hexane extracts of banana extract showed the antibacterial potency of four different extractsranging 50-80 mg/ml. The acetone and methanol extracts of all banana peel extracts showed similar zones of inhibition activity at10 mg/ml concentration against Gram negative bacteria rangingfrom 7 to 8.5 mm whereas no difference was seen in the case of Gram positive bacteria [23]. Similar findings were reported in chloroform and ethyl acetate of banana peel extracts [24, 25].
Ananas comosus (pineapple) crown leaves: In the case of *Ananas comosus*, their different extracts (methanol, ethanol, chloroform and n-hexane) did not exhibit significant zone of inhibition against *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. *Zea mays (maize) husk:* The antibacterial activity of *Zea mays* (Fig, 2A-B) was highest in methanolic and ethanolic extracts. The ethanol extract (80 mg/ml) exhibited the maximum inhibition activity (±10 mm) against *Escherichia coli* as compared to otherextracts like methanol (±7 mm), chloroform (±5 mm) and hexane (±5 mm) in case of *E. coli, B. subtilis* and *P. aeruginosa*. In case of methanolic extract 80 mg/ml concentration would be the mosteffective in *Escherichia coli*, as compared to other bacterial species *B. subtilis* and *P. aeruginosa*. In ethanolic, chloroform and n-hexane extracts higher zone of inhibition was against *E. coli* as compared to the other bacterial species *P. aeruginosa* and
B. subtilis at 80 mg/ml concentration (Fig. 2A -B). The medicinal properties of corn husk is supported by several authors as it

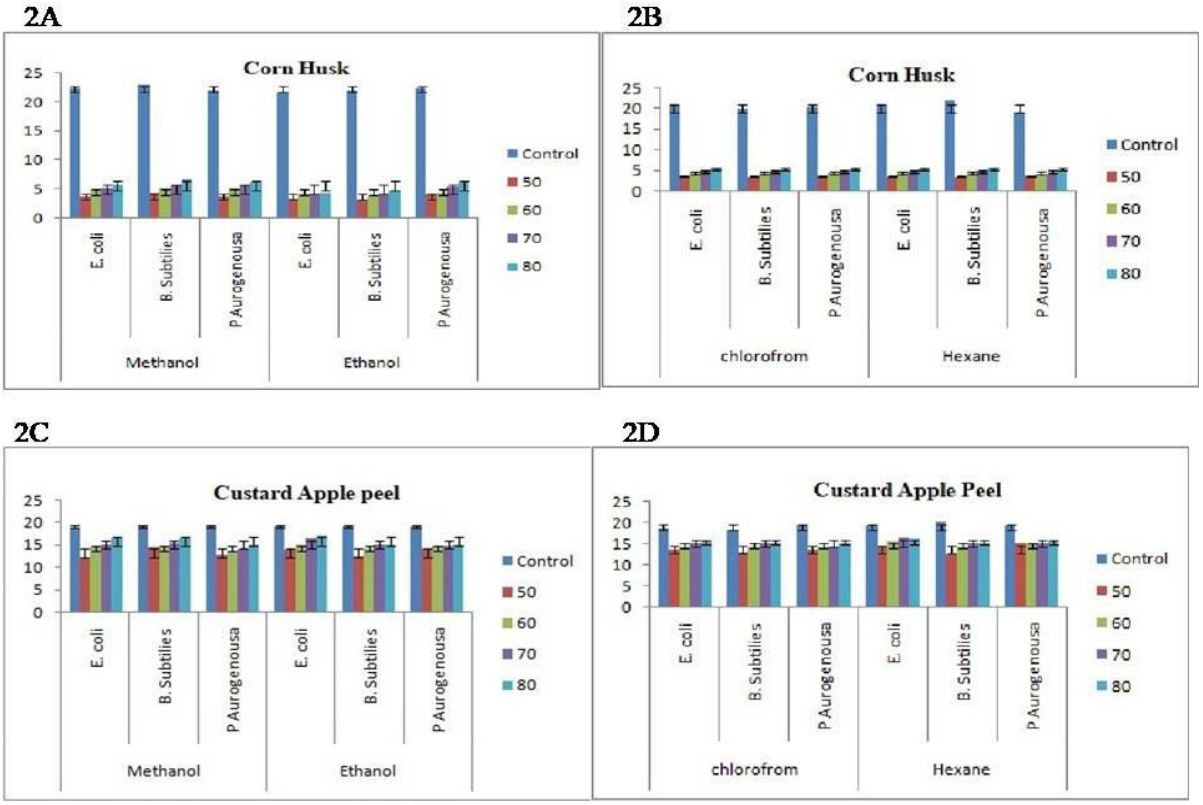

Figure 2. Structure of corn husk (A-B) and custard apple peel (C-D) methanol, ethanol (polar solvent), chloroform and hexane (non-polar solvent) extracts.

exhibited antioxidant activity [26], anti-diabetic activity [27], antibiotic activity towards corn earworm, resistance to insect attacks and antitumour activity [28]. Surjee and Zwain [29] reported the inhibitoryeffect of crude aqueous and ethanolic extracts of husk of *Zea mays* against bacterial isolate. The aqueous and ethanolic extractexhibited higher antibacterial activity against Gram negative and Gram positive bacteria. The ethanolic extracts acted as better antibacterial agents than the aqueous extracts, at concentrations of 50, 25 and 12.5 %. A zone of inhibition in the conc. till reach toheight of 20 mm to aqueous extracts and 38 mm to ethanolic extracts.

Annona reticulata peel: The highest antibacterial activity was observed in methanolic and ethanolic extracts (80 mg/ml) of *Annona reticulata* peel (Fig. 2C-D). The methanolic extract exhibited the maximum inhibition activity of ±17 mm against *E. coli* comparedto other extracts like ethanol ±16 mm, chloroform ±15 mm and hexane ±14 mm in case of *E. coli, B.subtilis* and *P. aeruginosa*.Ethanolic extract 80 mg/ml showed (Fig. 2A-B) maximum zone of inhibition in *E. coli* against *P. aeruginosa* and *B. subtilis*. In case of chloroform and n-hexane extract, 80 mg/ml concentration was more effective against *E. coli* as compared to *B. subtilis* and *P. aeruginosa* (Fig. 2 C-D). The extract was effective against *E.coli, B.subtilis* and *P. aeruginosa* but inefective against *S. aureus* and *S. typhi* [30]. Gowdhami [31] reported anti-bacterial activity for different microbial isolates. Aqueous extract of custard apple exhibited antimicrobial activity against *E.coli* and *S. paratyphe* at ±16 mm and ±14 mm, respectively. *A. muricata* extract has a wide activityrange against microbes responsible various bacterial diseases [31].

Phytochemical assessment of peel extracts: Phytochemistry of traditionally valued fruits and fruit waste products is essential toknow as this may lead to the discovery of new medicine of therapeutic importance. Different phytochemical tests were performed with different extracts like methanol, ethanol, chloroform and hexane of the peels of *Punica granatum, Citrus limetta, Musa paradisiaca*, *Annona reticulata*, *Ananas comosus* crown leaves and *Zea mays* husk. In present study we revealed the presence of carbohydrates, reducing sugars, deoxy sugars, protein, sterols, glycosides,phenolic compounds, tannins, saponins and flavonoids (Table 1). Similar findings have been reported by other workers [14, 19, 20]. A recent study on the phytochemical analysis has reported the presence of tannins (punicalin and punicafolin), flavonoids, glycosides, including luteolin and apigenin in the peels of *Punica granatum, Citrus limetta, Musa paradisiaca*, *Annonareticulata, Ananas comosus* crown leaves and *Zea mays* husk. The occurrence of gallotannins in the peels has also been reported[15, 16]. Tannins prevent the microbial growth by precipitating microbial proteins. Secondary metabolites like flavonoids are synthesized by fruit peel in response to microbial infection. The growth of many molds, yeasts, bacteria and viruses are inhibited by tannins [17]. In nature, there are a large number of different types of antimicrobial compounds that play an important role in the natural defence of all kinds of living organisms. Fruit waste extracts are some of the most commonly used natural antimicrobial agents in foods. Custard apple, recently described as nature's power fruit, is a plant used in medicine for the treatment of various infectious diseases.

Table 1. Phytochemical analysis of different fruit waste by different extract.

Plant extract	Molisch test	Fehling test	Barfoed	Benedict	wagner	Seliwanoff's test	Bial test	Ninhdrin test	Phenol	Mayer	Saponins	Tannins test	Quinone	Terpenoid	Steroid
Pomegranate peel methanol	++	++	++	++	--	++	--	++	++	--	--	++	++	++	++
Pomegranate peel ethanol	++	++	++	++	--	++	--	++	++	--	--	++	++	++	++
Pomegranate peel chloroform	++	++	++	++	--	--	++	--	++	++	--	++	++	++	++
Pomegranate peel hexane	++	++	--	++	--	++	++	++	++	++	--	++	++	++	++
Banana peel methanol	++	++	++	++	--	++	++	++	++	--	--	++	++	++	++
Banana peel ethanol	++	++	--	++	--	++	++	++	++	--	--	++	++	++	++
Banana peel chloroform	++	++	++	++	--	++	++	++	++	--	--	++	++	++	++
Banana peel hexane	++	++	--	++	--	++	++	--	++	--	--	++	++	++	++
Sweet lime peel methanol	++	++	++	++	--	++	--	++	++	++	--	++	++	++	++
Sweet lime peel ethanol	++	++	++	++	--	++	--	++	++	++	--	++	++	++	++
Sweet lime peel chloroform	++	++	++	++	--	++	++	++	++	--	--	++	++	++	++
Sweet lime peel hexane	++	++	--	--	++	++	++	--	++	--	--	++	++	++	++
Pine apple leaves methanol	++	++	++	++	--	++	++	++	++	--	--	++	++	++	++
Pine apple leaves ethanol	++	++	++	++	--	++	++	++	++	--	--	++	++	++	++
Pine apple leaves chloroform	++	++	--	--	++	--	++	--	++	--	--	++	++	++	++
Pine apple leaves hexane	++	++	--	--	++	--	++	--	++	--	--	++	++	++	++
Corn husk methanol	++	++	++	++	--	++	--	++	++	--	--	++	++	++	++
Corn husk ethanol	++	++	++	++	--	++	--	++	++	--	--	++	++	++	++
Corn husk chloroform	++	++	++	++	--	++	--	++	++	--	--	++	++	++	++
Corn husk hexane	++	++	--	--	++	--	++	++	++	++	--	++	++	++	++
Custard apple peel methanol	++	++	++	++	--	++	--	++	++	--	--	++	++	++	++
Custard apple peel ethanol	++	++	++	++	--	++	--	++	++	--	--	++	++	++	++
Custard apple peel chloroform	++	++	--	++	--	++	++	--	++	++	--	++	++	++	++
Custard apple peel hexane	++	++	--	++	--	--	++	--	++	++	--	++	++	++	++

IV. Conclusions

We used polar and non-polar components in the current work with both Gram positive (Bacillus subtilis and Pseudomonas aeruginosa) and Gram negative (Escherichia coli) bacteria. The peels of Punica granatum, Citrus limetta, Musa paradisiaca, Annona reticulata, and Zea mays husk displayed good antibacterial efficacy as did the extracts made from methanol, ethanol, chloroform, and n-hexane. Future research will incorporate further findings that support the use of various extracts for the treatment of infectious disorders. The extracts are also abundant in a number of phytochemical elements, including sterols, glycosides, phenolic compounds, tannins, saponins, flavonoids, and proteins.

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