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Evaluation of anti-bacterial and anti-fungal activity of ferulic acid-An isolated phenolic compound from pineapple peel

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Abstract
Plants have the ability to synthesize a wide variety of secondary metabolites having a capacity to inhibit the pathogens growth. The present study appraise the antibacterial and antifungal activity of ferulic acid- an isolated phenolic compound from pineapple peel against four different bacteria namely *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* and five pathogenic fungi such as *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus niger*, *Colletotrichum gloeosporioides* and *Candida albicans* using agar well diffusion method. Different concentrations of ferulic acid ranging from 250 µg to 1000 µg were tested against the selected plant pathogenic fungi whereas ferulic acid 500µg and 1000µg were evaluated against gram positive and gram negative bacteria and the inhibition zone were compared against standard drug Ciprofloxacin (2µg) and Clotrimazole (100µg) respectively as positive control. Ferulic acid showed maximum potentiality against *Bacillus cereus* - a gram positive bacteria and with the most common fungal plant pathogens namely *Colletotrichum gloeosporioides*. Results from the study showed that the ferulic acid isolated from the pineapple waste has significant antimicrobial activity against harmful pathogenic microorganisms. This biologically active phenolic compound can be further studied to develop new antimicrobial drug.

Keywords: pineapple peel, antibacterial, antifungal, ferulic acid, agar well diffusion method

Introduction
The tropical edible fruit pineapple (*Ananas comosus* (L.) Merr belongs to Bromeliaceae family is widely used for juice extraction in industry which produce wastes mainly as peel and its eco-friendly utilization is necessary. The development of new anti-bacterial compound is essential because of the indiscriminate practise of synthetic antibiotics cause a prolonged increase in antibiotic resistance in microorganism (Chopra *et al*., 1997) [1]. Plants are the valuable source of natural products for maintaining human needs and utilization of compounds from plants for pharmaceutical purposes heighten in our country. The usefulness of plant chemicals having well known antimicrobial potentiality is powerful in the therapeutic treatments. According to Agrios, (2018) [2] different pathogenic fungi during their various developmental stages produce vegetable and fruits with low nutritional value, organoleptic characteristics, limited shelf life. Normally, the plant pathogenic fungi are controlled by synthetic fungicides, but its usage become restricted due to the adverse reaction of pesticides towards human wellness and environment (Harris *et al*., 2016) [3]. The higher production of agrochemicals and appearance of resistant pathogens to the employed products leads the exploration of new active molecules and control methods. Development of natural antifungal compounds is important against microbial diseases in plants, usage of compounds derived from plants for antimicrobial treatment become popular day by day (Carvalho *et al*., 2018) [4]. Limited number of studies were conducted on antifungal potentiality of compounds derived from fruit waste. The current work analyse the antibacterial and anti-fungal potential of ferulic acid – an isolated phenolic compound from pineapple waste against pathogenic bacteria and fungi. Further studies are warranted to develop new antimicrobial drugs using this biologically potent phenolic compound.

Materials and Methods
Plant material
The peel of Mauritius variety of pineapple fruit (the most popular cultivar grown in Kerala) collected from Pineapple Research Station, Vazhakulam, Muvattupuzha, which is the main cultivation region of pineapple in Kerala, India. The plant material was preserved as herbarium specimen in the Post Graduate and Research Department of Botany, Christian College, Kattakada Herbarium having specimen number HCCK 108 as reference material.

Antibacterial activity
Antibacterial activity of phenolic compound Ferulic acid isolated from pineapple peel (500µg and 1000µg) were analysed contrarily with strains of bacteria named *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli*

(ATCC 25922), *Staphylococcus aureus* (ATCC 33591) and *Bacillus cereus* (ATCC 10876) by agar well diffusion mode (Javed *et al*., 2011) [5]. Bacterial strains brought from National Collection of Industrial Microorganism (NCIM), Pune, India. The Potato Dextrose Agar (Himedia, Mumbai, India) plates prepared by pouring autoclaved agar into sterile petri plates and were inoculated with 0.1% inoculum suspension of each test bacteria. Three wells of 10 mm diameter were generated in each plate with the help of a sterile well borer. 500 µg, 1000µg Ferulic acid were loaded in two wells and the positive control 2 µg Ciprofloxacin loaded in the third well. Incubation period was 24 h at 37ºC, The experiment done in triplicates, the average diameter for zone of inhibition was calculated in millimeters. A simple statistical analysis was carried out to calculate the standard deviation (Halawi *et al*., 2015) [6].

Antifungal activity

Anti-fungal activity for Ferulic acid isolated from pineapple peel was analysed against fungal strains such as *Alternaria alternata* (ATCC 20084), *Fusarium oxysporum* (ATCC 48112), *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 16404), and *Colletotrichum gloeosporioides* (ATCC 20980) by the method agar well diffusion (Javed *et al*., 2011) [5]. Strains of fungi were collected from National Collection of Industrial Microorganism (NCIM), Pune, India. The Potato Dextrose Agar (Himedia, Mumbai, India) plates were made, 0.5% inoculum suspension of the test organisms were inoculated in each plate, four wells of 10 mm diameter were made with the help of a sterile well borer. 250 µg, 500 µg, 1000 µg Ferulic acid were loaded in three wells and 100 µg standard antifungal drug Clotrimazole (positive control)was loaded in the fourth well. Incubation period allowed was 24 h at 37ºC. The inhibition zone was measured and each experiment done in triplicates, The average diameter of zone of inhibition was calculated in millimeters. A simple statistical analysis was carried out to calculate the standard deviation. (Halawi *et al*., 2015) [6].

Results and Discussion

Exploration of antibacterial plant products have mechanism of action differ from conventional chemical drug was analysed using natural phenolic compound namely ferulic acid isolated from the pineapple peel on preferred bacterial and fungal strains (Table 1). The antibacterial potentiality of ferulic acid exhibited varied susceptibility against *Escherichia coli, Klebsiella pneumonia, Bacillus cereus and Staphylococcus aureus* at the concentrations tested (Figure 1 a-d) shows antibacterial potential of the phenolic compound namely ferulic acid visualized as zone of inhibition. Ferulic acid showed inhibitory effects against the all four tested bacterial strains. Among the pathogens tested *Bacillus cereus and Staphylococcus aureus* were the most resistant species. The increased bactericidal activity were observed by 1000 µg of ferulic acid against *Bacillus cereus*, which showed zone of inhibition about 30 ± 0.06 mm followed by *Staphylococcus aureus* which showed zone of inhibition about 24 ± 0.05mm. The synthetic antibiotic Ciprofloxacin exhibit antibiosis and was compared against all the tested bacteria.

Table 1: Bactericidal potentiality of ferulic acid against selected bacterial strains.

Bacterial Strains	Zone of Inhibition (mm)		
	Ferulic acid (500µg)	**Ferulic acid (1000 µg)**	**Ciprofloxacin (2 µg)**
Klebsiella pneumoniae (ATCC 700603)	12± 0.15	16 ± 0.12	21 ± 0.07
Escherichia coli (ATCC 25922)	13 ± 0.04	15 ± 0.10	0
Staphylococcus aureus (ATCC 33591)	16 ± 0.02	24 ± 0.05	21 ± 0.14
Bacillus cereus (ATCC 10876)	18 ± 0.08	30 ± 0.06	30 ± 0.04

Values of zone of inhibition in mm are mean ± SD of three independent replications; 0 = No zone of inhibition.

Zones of inhibition for the highest dose of Ferulic acid (1000µg) evaluated was 26 ± 0.32, 22 ± 0.24, 25 ± 0.32, 39 ± 0.40 and 27 ± 0.33 mm respectively for *Alternaria alternate, Fusarium oxysporum, Aspergillus niger, Colletotrichum gloeosporioides* and *Candida albicans* in turn exhibited more or less similar inhibitory activity with the positive control Clotrimazole (100µg) (Table 2). The lower dose of Ferulic acid (250µg) exhibited relatively lower inhibitory activity against all the fungal strains studied. Interestingly, the most common plant pathogenic fungus, *Colletotrichum gloeosporioides* employed was most susceptible to all the concentrations of Ferulic acid used, of which the highest dose (1000µg) of the phenolic compound showed significant inhibitory activity (39 ± 0.40) which was higher than the standard antifungal drug used (34 ± 0.39) (Figure 2 a-e).

Plant derived phenolic compounds are safe for human consumption, Their lipophilic nature attract the lipid molecules present in bacterial cell membranes and destroy its cell wall so phenolic compounds are used to prevent the growth of food spoilage microorganisms. (Maurer, 2001) [7]. The anti-bacterial determination of pineapple peel waste in four crude extracts against gram positive as well as gram negative bacterial strains reported by Lubaina et al. (2019) [8] resulted significant anti-bacterial potential of this thrown away waste. The studies done by Thanish *et al.* (2016) [9] revealed the antimicrobial activity of pineapple extract against *Streptococcus mutans* similarly Zharfan *et al.*, 2017 [10] reported pineapple extract showed increased antimicrobial potentiality against gram negative bacteria *Pseudomonas aeruginosa*. The bactericidal potentiality of a compound is defined by their ability to reach at the site of action in bacterial cell wall. The phenolic compound may inhibit the Electron Transport chain by interfering with the redox reactions (Franks and Leib,

1986) [11]. Thus, Ferulic acid being more hydrophobic may reveal its greater anti-bacterial activity. Diseases caused by pathogenic fungi leads to reduction in the yield of crop plants which promote the researchers to find out new natural antifungal drugs with minimum side effects (Denning and Bromley, 2015) [12]. Lopez-garcia *et al*., (2012) [13] studies showed bromelain the enzyme found in pineapple having immense anti-fungal potentialities and is an inhibitor of fungal plant pathogens. Its antifungal activity is related to the proteolytic activity of this protease. Present study revealed the significant antifungal and anti-bacterial potentialities of Ferulic acid which in turn provide the use of these phenolic compound in various aspects.

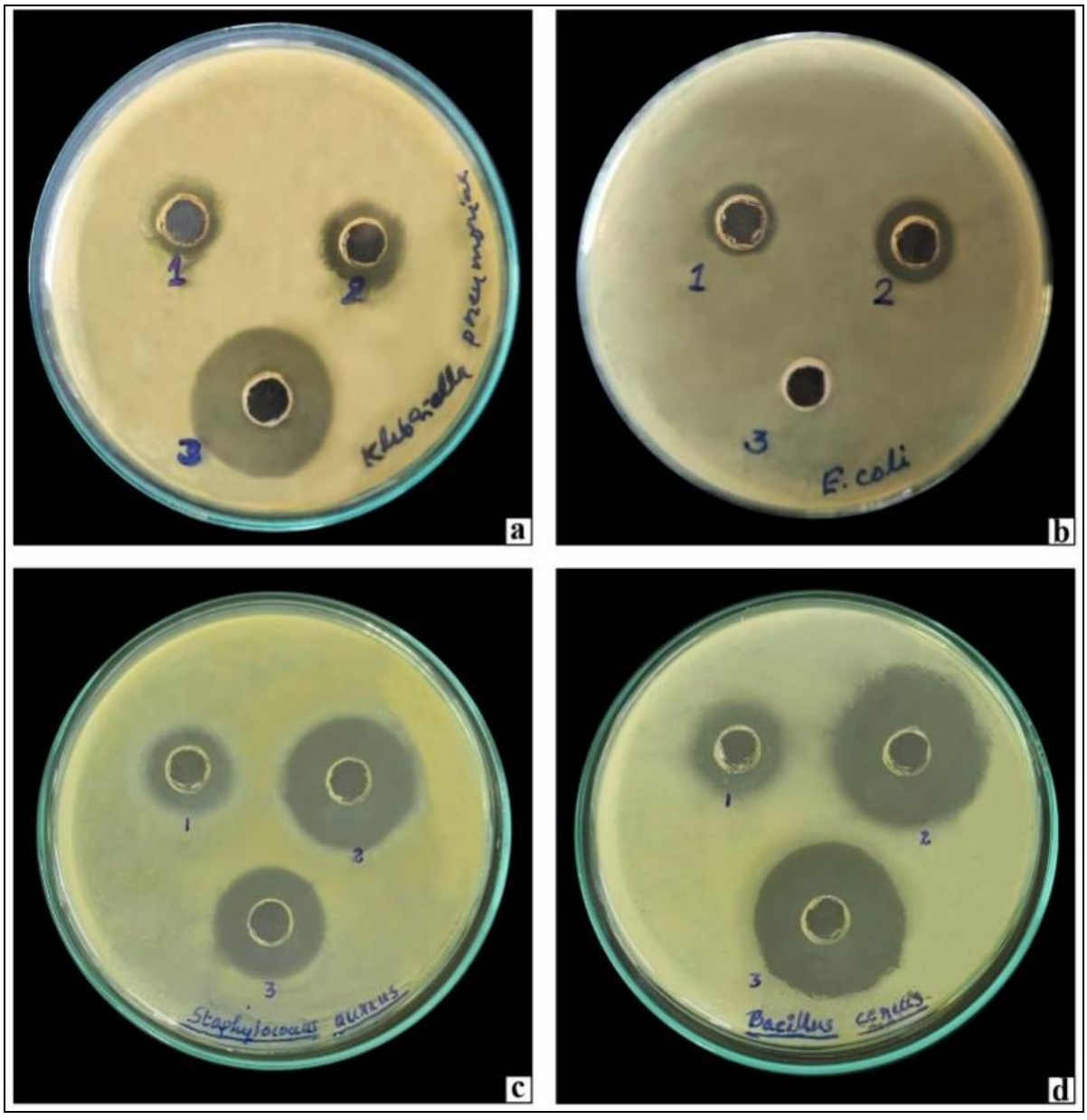

Fig 1(a-d): Anti-bacterial activity of from pineapple peel against a. Klebsiella pneumoniae. b. Escherichia coli c. Staphylococcus aureus d. Bacillus cereus 1. 500ug FA, 2.1000ug FA, 3. Ciprofloxacin

Table 2: Anti-fungal activity of ferulic acid against fungal strains.

Fungal Strains	Zone of Inhibition (mm)			
	Ferulic acid (250µg)	**Ferulic acid (500µg)**	**Ferulic acid (1000µg)**	**Clotrimazole (100µg)**
Alternaria alternata	13 ± 0.10	19 ± 0.14	26 ± 0.32	30 ± 0.44
Fusarium oxysporum	11± 0.09	15 ± 0.12	22 ± 0.24	12 ± 0.11
Aspergillus niger	11± 0.08	13± 0.12	25 ± 0.32	26 ± 0.34
Colletotrichum gloeosporioides	24 ± 0.27	28 ± 0.34	39 ± 0.40	34 ± 0.39
Candida albicans	13 ± 0.14	18 ± 0.16	27 ± 0.33	27 ± 0.09

Values of zone of inhibition in mm are mean ± SD of three independent replications

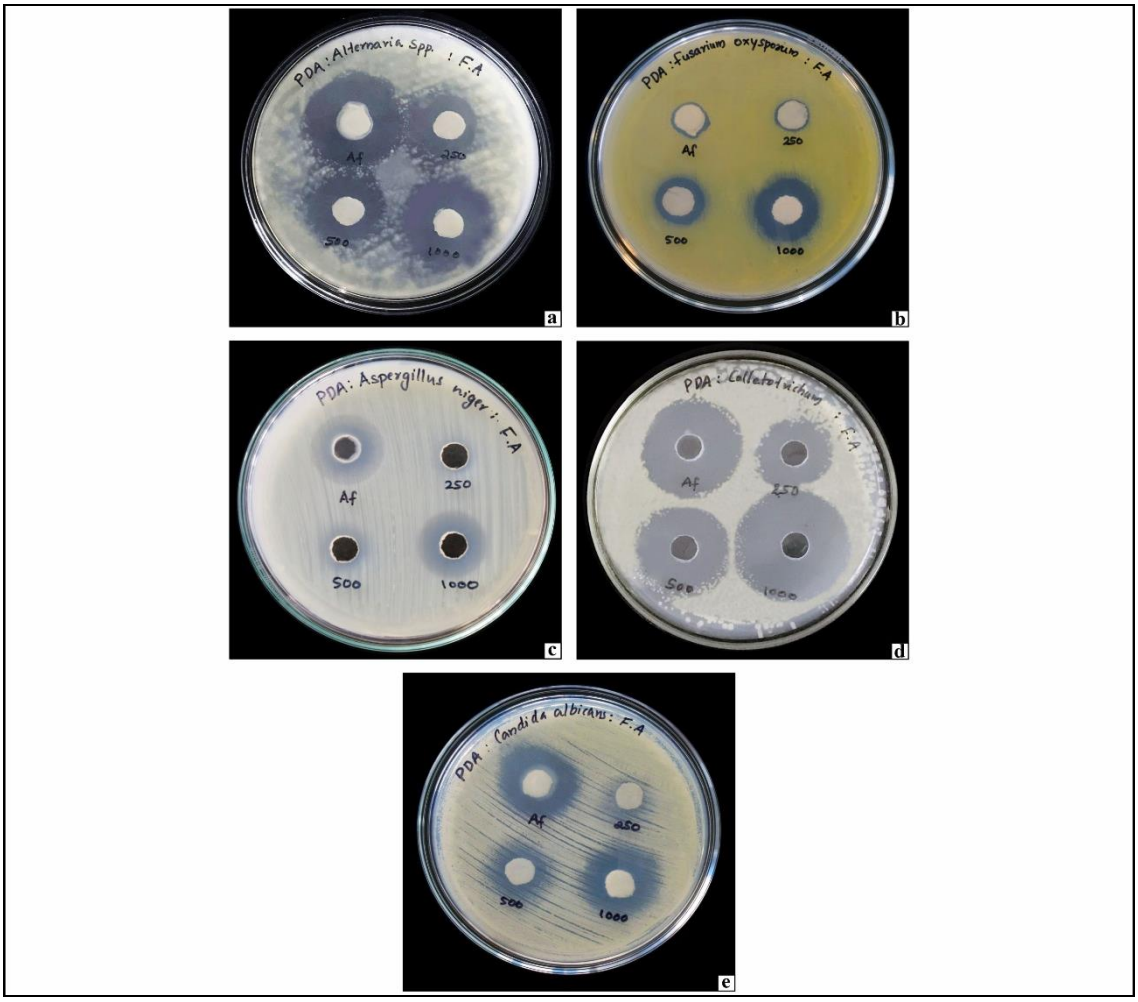

Fig 2 (a-e): anti-fungal activity of ferulic acid (FA) isolated form pineapple peel aginst a. Alternaria alternata b. Fusarium oxysporum c. Aspergillus niger d. Colletotrichum gloeosporioides e. Candida albicans Af-100ug Clotrimazole, 250ug FA, 1000ug FA

Conclusion

Antimicrobial potential exhibited by Ferulic acid isolated from pineapple peel waste was effectual in case of gram-positive as well as gram-negative strains of bacteria and also to selected plant fungal pathogens. From the results it can be concluded that isolated phenolic compound from the pineapple peel is beneficial for production of antibiotic and other potential applications in medicine. Different compounds from pineapple peel having antimicrobial potentiality come to great popularity due to their use as natural supplement to replace manufactured antimicrobials with natural ones. The phenolic compound found to be effective against all the pathogens used in this study, which highlight the potential of herbal drugs against bacteria and fungi and their possible use as local medicine.

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