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EVALUATION OF ANTIBACTERIAL ACTIVITY OF FIVE SELECTED FRUITS ON BACTERIAL WOUND ISOLATES

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ABSTRACT

The present study describes the antibacterial activity of five selected fruits on four pathogenic bacteria isolated from patients suffering with wound infections. Ethanolic extracts of fruits were more effective than aqueous extracts. *Punica granatum* has shown highest antibacterial activity followed by *Malus domestica, Citrus limonium* and *Citrus limetta*. Lowest antibacterial activity was recorded for *Solanum lycospersicum*. Among all bacteria tested highest antibacterial activity was recorded against *S. aureus* and least was recorded for *K. pneumoniae*.

KEY WORDS : Antibacterial activity, Punica granatum, Malus domestica, Citrus limonium, Citrus limetta, Solanum lycospersicum, Agar well diffusion method.

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INTRODUCTION

Fruits are one of the oldest forms of food known to man. There are many references to fruits in ancient literature. Vedas state that fruits form the base of the Food of Gods. According to Qur'an, the fruits like grapes, date, fig, olive and pomegranate are gifts and heavenly fruits of God. The people in ancient times regarded fruits to be endowed with magic or divine properties. World health organization has listed over 21,000 plant species used around the world for medicinal purposes. In India about 2500 plant species belonging to more than 1000 genera are being used in indigenous system of medicine which symbolizes the rich tradition for herb and herbal remedies[1]. From the ancient time different cultures around the world have used herbs and plants as a remedy in different diseased condition and maintain health. Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material. Scientific studies available on medicinal plants indicate that promising phytochemicals can be developed for many health problems [2]. Phytochemicals are becoming increasingly known for their antioxidant activity [3]. Especially Flavonoids (flavonoids) are a group of natural products with many biological and pharmacological activities like antibacterial, antiviral, antioxidant, anti-inflammatory and antimutagenic effects etc., [4, 5].

There is a great demand of fruit juices in treatment of various illnesses such as arthritis, heart diseases , muscle aches and drug addiction [6]. Even though thousands of antimicrobial compounds exist, the ability of microbes to develop resistance to even the most powerful antimicrobial compounds is amazingly rapid [7]. Plant based extracts can be extracted from any part of plant like barks, leaves, fruits, seeds and fruit rinds etc., [8]. The antimicrobial activity of various fruit extracts have been tested against different bacteria and fungi [9]. Generally Gram negative bacteria are more resistant than Gram positive bacteria [10, 11, 12].

Pomegranate (*Punica granatum* L.) fruits are widely consumed, fresh and in commercial products, such as juices, jams and wines. Most pomegranate fruit parts are known to possess enormous antioxidant activity [13]. The flower [13], seed oil [14], seed extract and peel extract [15] of pomegranate also have a potent antioxidant activity. Pomegranate belongs to punicaceae family. The edible part of fruit contains considerable saccharides, polyphenol and important minerals. The major class of pomegranate phytochemicals is the polyphenols (phenolic rings bearing multiple hydroxyl groups) that predominate in the fruit. Pomegranate polyphenols include flavonoids (flavonols, flavanols and anthocyanins), condensed tannins (proanthocyanidins), and hydrolysable tannins (ellagitannins and gallotannins) [16]. Apple (*Malus domestica*) is a worldwide diffused fruit with many health beneficial effects, mostly due to the presence of phenolic compounds. Apples also ranked second for total content of phenolic compounds, including quercetin, catechin, phloridzin and chlorogenic acid, all of which are strong antioxidants, and thus capable of counterbalancing free radical activities that may cause cell injuries [6, 17]. The Phytochemical composition varies greatly with the different varieties of apples and there by helps in developing new antimicrobials against various infectious diseases [17, 18, 19].

Citrus fruits are acidic fruits which contain healthy nutritional content that works wonders for the body. It acts as a fabulous source of vitamin C and a wide variety of essential nutrients required by the body. Fresh fruits and their hand-squeezed or industrially processed juices contain mostly flavanones and flavones [20, 21]. There are several Citrus species, of these common are *C. limonium* (lemon), *C. aurantium* (bitter orange), *C. limetta* (sweet lime) and *C. paradise* (grape fruit). Lemon (*Citrus limonium*) is an important medicinal plant of the family Rutaceae. It is cultivated mainly for alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (viz., leaves, stem, root and flower) of lemon against clinically significant bacterial strains has been reported [22]. Sweet limes are excellent source of free citric acid, natural sugar, vitamin C, calcium and phosphorus. They contain by far more vitamin C than the lemon. Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, anticancer and antiviral activities [23, 24]. Literature suggests that the fruit of *C. aurantium* possess antianxiety activity [25] and antiobesity activity [26]. Peel of *C. limonium* possesses cytotoxic[27] and antimicrobial activity [28], fruit of *C.limonium* and *C.limetta* possesses antioxidative stress [29] and antiurinary lithogenesis [30].

Tomatoes (*Solanum lycospersicum*) are now eaten freely throughout the world, and their consumption is believed to benefit the heart, among other organs. They contain the carotene lycopene, one of the most powerful natural antioxidants. In addition to its flavor properties, tomatoes are reported to possess numerous beneficial nutritional and bioactive components that may also benefit human health. These include the nutrients vitamin A, vitamin C, iron, and potassium; nonnutritive digestible and indigestible dietary fiber; the antioxidative compounds lycopene, *β*-carotene, and lutein [31, 32]; and the cholesterol lowering [33,34]and immune system enhancing glycoalkaloids tomatine and dehydrotomatine[35]. Consumption of tomatoes, tomato products, and isolated bioactive tomato ingredients is reported to be associated with lowered risk of cancer [36], heart disease [37], diabetes [38] and hypertension [39]. These considerations suggest that edible tomato contains antimicrobials which may have multiple benefits.

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms have increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [40]. Keeping in view this adaptive nature of bacteria and drawback of standard antibiotics , current work was undertaken with following objectives: (1) to evaluate the antibacterial activity of five selected fruit extracts on clinical bacterial strains isolated from patients suffering from wound infections by Kirby- Bauer's sensitivity testing technique and determination of minimum inhibitory concentration (MIC) (2) to find out effective fruit extract with highest antimicrobial activity which can be given to patients for medicinal use as probable substitute or along with standard antibiotics.

MATERIALS AND METHODS

Isolation and characterization of Test organisms

Sterile swabs sticks were used to collect surface and deep samples from patients with wound infections. Samples obtained with swab sticks were suspended in sterile peptone water to resuscitate infective microorganisms according to the modified Kirby- Bauers sensitivity testing technique [41, 42]. Ten-fold dilution of the suspension was made in peptone water and aliquot portion (0.1ml) inoculated onto surface dried fresh Nutrient and MacConkeys agar media. Inocula were spread evenly and incubated at 37^0C for 24-48 hrs [42, 43, 44]. Isolates were characterized [42, 44, 45 & 46] and pure cultures were identified by macroscopic, microscopic, physiological, biochemical methods [41, 47] and preserved on slants at 4^0C.

Preparation of bacterial inoculum

For inoculum preparation density of wound isolated bacterial cultures was adjusted equal to that of 0.5 McFarland standards (1.5 x 10^8 CFU/ml) by adding sterile distilled water. McFarland standards are used as a reference to adjust the turbidity of microbial suspension so that number of microorganisms will be within a given range. For the preparation of the 0.5 McFarland standard, 0.05ml of barium chloride ($BaCl_2$) (1.7% w/v $BaCl_2.2H_2O$) was added to 9.95ml of 0.18 M H_2SO_4 (1.0% w/v) with constant stirring. The McFarland standard tube was tightly sealed to prevent loss by evaporation and stored for up to 6 months. To aid comparison the test and standard were compared against a white background with a contrasting black line [48].

Evaluation of fruits for their antibacterial activity

Collection of fruits

Fresh fruits were purchased and collected from a well known market in Hyderabad city. Different fruits used in the study are Pomegranate (*Punica granatum*), Lemon (*Citrus limonium*), Apple (*Malus domestica*), Tomato (*Solanum lycospersicum*) and Sweet lime (*Citrus limetta*). All fruits were brought to lab, washed in running tap water; surface sterilized with 70% alcohol and then rinsed with sterile distilled water.

Phytochemical extraction method

For extraction, surface sterilized fruits as mentioned above were dried in an oven at 50^0C for 48 hrs followed by grinding into a fine powder. The powdered material was stored in air tight jars in refrigerator at 4^0C [49]. Two extractants i.e., water and ethanol (95%) were used for the phytochemical extraction of five fruits.

a) For extraction with water, 25g of powdered material was dissolved in enough sterilized distilled water to make up 100ml of aqueous extract (25% w/v). The mixture was kept undisturbed at room temperature for 24 hrs in a sterile flask covered with aluminium foil to avoid evaporation and subjected to filtration through sterilized Whatman no.1 filter. After filtration, the extract was evaporated in water bath until 25ml extract was left in the container.
b) For extraction with ethanol, 25g of powdered plant material was dissolved in enough ethanol to make 100ml of ethanolic extract (25% w/v). The extraction procedure was same as used for aqueous extract.

Agar well diffusion method

The antibacterial activity of 10 crude extracts (aqueous and ethanolic) of 5 fruits against four wound isolates was evaluated by using agar well diffusion method [50, 51]. PCA plates were inoculated with 100µl of standardized inoculum (1.5 x 10^8 CFU/ml) of each bacterium (in triplicates) and spread with sterile swabs. Wells or cups of 8 mm size were made with sterile borer into agar plates containing the bacterial inoculum and the lower portion was sealed with a little molten agar medium. 100µl volume of the fruit extract was poured into a well of inoculated plates. Sterilized distilled water or solvent (ethanol) was used as a negative control which was introduced into a well instead of fruit extract. Parallel with fruit extracts, the antibacterial activity was also analyzed with 8 commercially available standard antibiotics (ampicillin, benzyl-penicillin, streptomycin, ciprofloxacin, gentamycin, erythromycin norfloxacin and tetracycline) for comparative study. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extracts into the agar [52]. After incubation for 24 hrs at 37^0C, the plates were observed. If antibacterial activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the fruit extract. The diameter of inhibition zone (DIZ) was measured and expressed in millimetres. The mean values of the diameter of inhibition zones were calculated.

Determination of the minimum inhibitory concentration (MIC)

Extracts which exhibited high activities against one or several pathogenic wound isolates were further assayed for their minimum inhibitory concentration (MIC). This was carried out by the two fold serial dilution of the tested extracts in nutrient broth (2ml volumes), then inoculated with 100µl inoculum size with the test organisms. The alcoholic and aqueous crude extracts were prepared at concentrations of 10; 5; 2.5; 1.3; 0.6; 0.3; 0.2 % (w/v). The MIC was determined by the broth dilution method [53]. Nutrient broth samples (10 ml) were inoculated with different concentrations of the crude extracts and with 100µl of active inoculum of bacterial wound isolates (1.5 x 10^8 CFU/ml) in tubes and incubated for 24hrs at 37^0C. The MIC was determined as the lowest concentration of the extract which inhibited the organism [54].

RESULTS

In the present work antibacterial effect of selected fruit extracts were studied on bacterial cultures isolated from patients suffering with wound infections with an objective for an effective treatment therapy keeping in view antibiotic resistant nature of bacteria. The bacterial wound isolates were characterized and identified by studying different properties as mentioned in materials and methods. The colonial and cell morphologies are shown in Table-1 and biochemical characteristics are shown in Table-2. The identification characteristics were cross checked with those of standard manual [41, 47]. Total of four bacterial pure cultures were isolated- three gram negative bacteria, *Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli* and one gram positive bacterium *Staphylococcusaureus*.

Table 1
Colonial and cell morphology of bacterial cultures isolated from wound samples

Colonial characteristics	Grams nature and morphology	Capsule	Spore	Motility	Flagellum	Probable Species
Smooth circular and golden yellow colonies	Gram positive oval cells in bunches	-	-	-	-	*Staphylococcus* sp
Smooth and shiny colonies with green pigments	Gram negative small single rods	-	-	+	+	*Pseudomonas* sp
Smooth moist shiny cream colonies	Gram negative small single rods	-	-	+	+	*Escherichia coli*
Moist and mucoid raised creamy colonies	Gram negative large rods in short chains	+	-	-	-	*Klebsiella* sp

Table 2
Biochemical characteristics of bacterial cultures isolated from wound samples

Cat	Oxi	Coag	In	MR	VP	Cit	Urease	NO_3	H_2S	Sugar Fermantation					Identity of isolates
										Glu	Suc	Lac	Mn	F	
+	-	+	-	+	-	-	-	+	+	+	+	+	+	+	*S. aureus*
+	+	+	-	+	-	+	+	+	+	+	-	-	+	-	*P.aeruginosa*
+	-	-	+	+	-	-	-	+	-	+	-	+	+	+	*E. coli*
+	-	-	-	-	+	+	$+^s$	-	-	+	+	+	+	-	*K.pneumoniae*

Cat- catalase; Oxi-oxidase; Coag-coagulase; In-indole; MR-methyl red; VP-voges proskaeur; Cit-citrate; NO_3-nitrate reduction; H_2S-hydrogen sulphide; Glu-glucose; Suc-sucrose; Lac-lactose; Mn-mannitol; F-fructose; s-slow reaction.

Antibacterial activity of aqueous and ethanolic extracts of five fruits - Pomegranate (*Punica granatum*), Lemon (*Citrus limonium*), Apple (*Malus domestica*), Tomato (*Solanum lycospersicum*) and Sweet lime (*Citrus limetta*) was studied. Agar well diffusion method was adopted for determining antibacterial potency. Quantitative evaluation of antibacterial activity was carried out by broth dilution method for active extracts revealed by agar well diffusion method and MIC was determined. For comparative study 8 commercially available antibiotics were also used for checking antibacterial spectrum (Table-3). All experiments were carried out in triplicates and mean values were considered.

Table 3
Antibacterial activities of commercially available standard antibiotics

Standard Antibiotics	Diameter of Inhibition Zone in mm			
	S. aureus	*P. aeruginosa*	*E. coli*	*K. pneumoniae*
Ampicillin	18.0	**R**	20.0	7.0
Benzyl Penicillin	10.0	**R**	12.0	14.0
Streptomycin	22.0	10.0	9.0	15.0
Ciprofloxacin	30.0	28.0	10.0	24.0
Gentamycin	24.0	12.0	9.0	15.0
Erythromycin	25.0	8.0	18.0	22.0
Norfloxacin	8.0	**R**	8.0	8.0
Tetracycline	31.0	20.0	25.0	18.0

R- Resistant

Results obtained for antibacterial studies reveal following findings. Both aqueous and ethanolic extracts of fruits exhibited antibacterial activity towards all four bacterial wound isolates, with more activity observed with ethanolic extracts. There was significant variation in the antibacterial activities (DIZ values) of different fruit extracts. The ethanolic extracts of pomegranate exhibited highest antibacterial activity among all five fruits used with diameter of inhibition zone (DIZ) values between 20 and 26 mm (Table – 4 and Figure-1). 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. The results for aqueous pomegranate extracts have shown some variation. High antibacterial activity was recorded on *K.pneumoniae,* (rather than *S. aureus*) with a DIZ value of 25 mm; followed by

S. aureus, 23 mm; *P. aeruginosa*, 21 mm and; *E. coli*, 17 mm.

Table 4
Antibacterial activity of Ethanolic and Aqueous extracts of selected fruits

Solvent	Fruit	*S. aureus*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*
		Diameter of Inhibition Zone (DIZ) in mm			
Ethanolic	**Pomegranate**	26.0	22.0	25.0	20.0
	Apple	20.0	16.0	15.0	10.0
	Lemon	20.0	18.0	20.0	15.0
	Sweet lime	10.0	12.0	10.0	10.0
	Tomato	10.0	6.0	8.0	5.0
Aqueous	**Pomegranate**	23.0	21.0	17.0	25.0
	Apple	18.0	12.0	13.0	9.0
	Lemon	15.0	20.0	11.0	10.0
	Sweet lime	8.0	9.0	8.0	9.0
	Tomato	8.0	4.0	6.0	4.0

Pronounced antibacterial activity was recorded with ethanolic extracts of apple with DIZ values between 10 and 20 mm. Apple exhibited high activity towards *S. aureus* with a DIZ value of 20 mm; *P. aeruginosa*, 16 mm; *E. coli*, 15 mm and *K. pneumoniae* 10 mm. Similarly aqueous extracts of apple showed high antibacterial effect on *S. aureus* with a DIZ value of 18 mm; followed by *E. coli*, 13 mm; *P. aeruginosa*, 12 mm and lowest activity was recorded for *K. pneumoniae*, 9 mm. The ethanolic extracts of lemon exhibited higher antibacterial activity than apple but less than pomegranate. DIZ values recorded for lemon showed some variations when compared with pomegranate and apple. Same type of high antibacterial effect was observed on two bacterial wound isolates, *S. aureus* and *E. coli* with a same DIZ value of 20 mm; followed by *P. aeruginosa*, 18 mm; and least on *K. pneumoniae*, 15mm. Whereas aqueous extracts of lemon showed different antibacterial effects with DIZ values ranging between 10 and 20 mm. High effect was recorded on *P. aeruginosa*, 20 mm; followed by *S. aureus*, 15 mm; *E. coli*, 11 mm and least on *K. pneumoniae*, 10 mm.

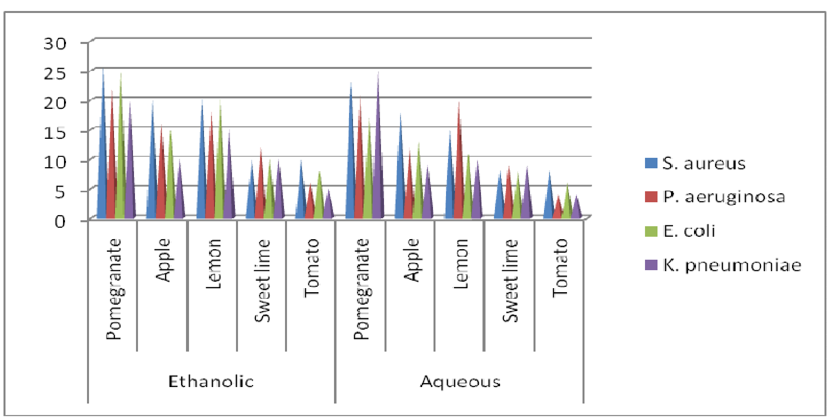

Figure 1
Diameter of inhibition zones (mm) of ethanolic and aqueous fruit extracts on bacterial wound isolates

Among all the five fruits used low antibacterial activity was recorded with fruits sweet lime and tomato. The DIZ values of ethanolic extracts of sweet lime range between 10 and 12 mm. Out of four bacterial isolates sweet lime showed more antibacterial effect on *P. aeruginosa* with a DIZ value of 12 mm and on remaining three bacterial cultures- *S. aureus*, *E. coli* and *K. pneumonia* antibacterial effect was observed with a same DIZ value of 10 mm. Aqueous extracts of sweet lime exhibited similar antibacterial with slight variation. For *P. aeruginosa* and *K. pneumoniae* DIZ value of 9 mm was recorded and for *S. aureus* and *E. coli,* 8 mm was recorded. The lowest antibacterial effect was observed with fruit tomato, both ethanolic and aqueous extracts of tomato showed more antibacterial effect on *S. aureus* with a DIZ value of 10 and 8 mm respectively, whereas the inhibitory effect on other bacterial isolates was comparatively less (Table-4 and Figure-1).

Quantitative evaluation of antibacterial activity (MIC) was carried out by broth dilution method for four fruits out of five as one of the fruit tomato revealed very low antibacterial effect by agar well diffusion method. Figure-2 shows the MIC of selected fruit extracts: *Punica granatum*, *Malus domestica*, *Citrus limonium*, and *Citrus limetta* on four bacterial wound isolates. A wide range of MIC values was recorded depending on the microbial strain. The MIC values of ethanolic extract of *Punica granatum* and *Citrus limonium* showed high activity against *S. aureus* and *E. coli* (0.6% w/v) and lowest activity was recorded against *K. pneumoniae* (2.5% w/v). *Malus domestica* ethanolic extracts exhibited high MIC values against *S. aureus* and *P. aeruginosa* with a value of 0.6% (w/v) and lowest activity against *K. pneumoniae* (2.5%). MIC values of aqueous extracts of *Punica granatum*, *Malus domestica* and *Citrus limonium* showed low activity compared with ethanolic extracts on all the four bacterial wound isolates, the least effect was observed for *K. pneumoniae*. The lowest MIC values were recorded for aqueous extracts of *Citrus limetta* (Figure-2).

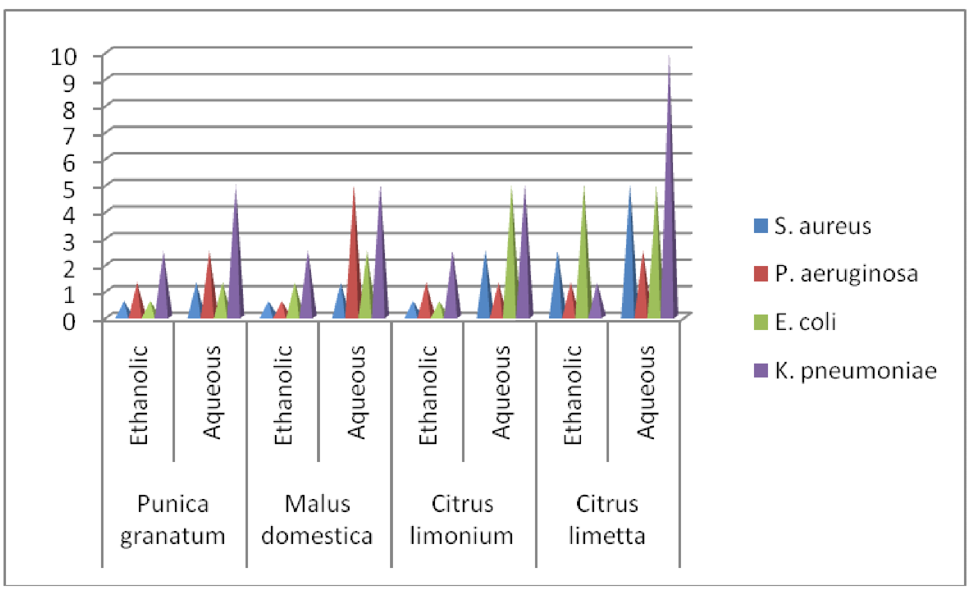

Figure 2
Minimum inhibitory concentration (MIC) w/v of selected fruit extracts on bacterial wound isolates

DISCUSSION

The emergence of drug resistance with patient's poor compliance, drugs adverse effects and the higher cost of therapy combinations, indicates a strong need for a therapy regimens with similar or higher antibiotics beneficial properties but with better adverse effects profiles. Results of the current study suggest a class effect antibacterial activity for clinical isolates, and indicate the superiority of the antibacterial activity of fruit extracts compared to standard antibiotics. Antibacterial activity of aqueous and ethanolic extracts of five fruits: Pomegranate (*Punica granatum*), Lemon (*Citrus limonium*), Apple (*Malus domestica*), Tomato (*Solanum lycospersicum*) and Sweet lime (*Citrus limetta*) was tested on four bacterial clinical isolates: *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus* from patients suffering with wound infections. Similar pathogenic organisms have been reported by Carter and Chengappa [41]; Subrahmanyam et al., [55]; Stepp and Woods [56]; Nester et al., [57]; Baron [58]; Mahon and Manuselis [59]; Talaro and Talaro [60] and Wiley et al., [61]. The antibacterial potency was initially determined by the agar well diffusion method (as shown in Table-4 and Figure-1) followed by quantitative evaluation of antibacterial activity by MIC method (as shown in Figure-2). Ethanolic extracts of all the fruits exhibited higher antibacterial effect than aqueous extracts.

Among all the fruits highest antibacterial activity was recorded with pomegranate fruit which inhibited all four wound isolates in following order- *S. aureus* > *E. coli* > *P. aeruginosa* > *K. pneumoniae*. Similar order of inhibitory antibacterial effect by pomegranate was also reported by Saad et al., [16] where in ethanolic extracts of pomegranate demonstrated high antibacterial activity than aqueous extracts. The antimicrobial effects of pomegranate were previously studied. Indeed, it is reported that the bark, leaves, flowers, and fruits of pomegranate are widely used as phytotherapeutic agents in Brazil [62]. Ahmad and Beg [50] reported that alcohol extracts of pomegranate fruits showed antibacterial activity when tested against *S. aureus*, *E. coli* and *Shigella dysentriae*. Prashanth et al. [63] also reported methanolic extracts of *Punica granatum* fruit to be active against all microorganisms tested in their study. These results are in accordance to results obtained in the present study for bacteria wherein antibacterial activity was observed for all the four bacterial cultures tested. Melendez and Capriles [64] have also reported that extracts from pomegranate fruits possess in vitro antibacterial activity against many bacteria tested showing an inhibition zones of 11-31 mm. Interestingly, they stated that in Puerto Rico, it is very common practice to use these plant extracts as remedies for colds and bacterial infections. Their results provide evidence for the presence of antimicrobial compounds in the crude methanolic extracts of these plants. These findings and the results obtained in our study clearly confirm the effectiveness of pomegranate fruit on inhibition of microbial activity. Ahmet Duman et al., [65] also reported the in vitro antibacterial activity of extracts obtained from six pomegranate cultivators against the bacteria *B. megaterium, P. aeruginosa, S. aureus, C. xerosis, E. coli, E. faecalis* and *M. luteus*, showing inhibition zones ranging from 13-26 mm.

After pomegranate, a good antibacterial effect was recorded for lemon which equally inhibited *S. aureus* and *E. coli* (20 mm) followed by *P. aeruginosa* and *K. pneumoniae* (18 and 15 mm respectively). The order of inhibition followed same pattern exhibited by pomegranate. Antimicrobial activity of lemon was studied by Ashok Kumar et al., [66] on five bacterial strains- *S. aureus, B. subtilis, E. coli, K. pneumoniae* and *S. typhi* with DIZ values range between 9 and 11 mm. They have reported high antibacterial activity in ethanolic extracts than aqueous extracts. The results obtained in our study also confirm high antibacterial effect in ethanolic extracts. In an another interesting study of clinical samples, the antibacterial effect of lemon extract was studied against nine *Pseudomonas aeruginosa* strains isolated from clinical samples by Adedji et al., [67]. In this study lemon extract inhibited all nine strains of *P. aeruginosa* isolated from ear swabs, wound samples, urine samples, blood, cerebrospinal (cerebrospinal) fluid and eye swab. In an another report by Gulay Kirbaslar et al.,[68], antibacterial activity of lemon was studied against *E. coli*, *S. aureus* and *K. pneumoniae* with DIZ values of 14, 13 and 12 mm respectively. A common observation which can be noticed in these reports is that *K. pneumoniae* is the organism which is least inhibited. Lemon antimicrobial activity was also studied by Maruti Dhanavade et al., [69] where in antibacterial effect was studied against *Pseudomonas aeruginosa, Salmonella typhimurium* and *Micrococcus aureus* by acetone, methanol and ethanol extracts. High antibacterial activity was recorded with ethanolic extracts thereby again giving a confirmation that ethanolic extracts exert potential antibacterial effect. The DIZ values obtained in our study were higher than all these previous reports which clearly show that lemon has strong antibacterial activity.

Apple exhibited intermediate antibacterial activity between pomegranate and lemon. The order of bacterial inhibition was: *S. aureus* > *P. aeruginosa* > *E. coli* > *K. pneumoniae*. This pattern of inhibition is similar to that exhibited by pomegranate with a slight variation with respect to *E. coli* and *P. aeruginosa*. Antimicrobial activity of tropical fruits- Apple, pomegranate, guava and orange was studied by Anshika Malaviya and Neeraj Mishra[70]. In their study aqueous and ethanolic extracts were used against four bacterial cultures- *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* with DIZ values range between 2 and 10 mm, interestingly the results report that aqueous extract of apple exhibited high antibacterial effect than ethanolic extracts. The DIZ values obtained in their work are quite inferior when compared with our study. In another study by Florida Fratianni et al.,[71] antimicrobial effect of two varieties of apples: organically produced and conventionally produced apples were tested against useful lactobacillus species (*Lb. Acidophilus, Lb. bulgaricus, Lb. casei, Lb. fermentum, Lb. rhamnosus*) and pathogenic microorganisms of agro-food or human interest, such as *B. cereus*, which determines several cases of food borne illness and the entero-haemorrhagic *E. coli* DSM 8579, that causes serious cases of food poisoning. None of apple extracts assayed gave positive results against the 6 strains of lactic acid bacteria. The conventional extracts determined the formation of inhibition zones, having a diameter of about 10mm, only against the three strains of *B. cereus* GN105, DSM 4313 and DSM 4384; a mere marked action was evident only against *B. cereus* GN 105 with the maximum of extracts tested (inhibition zone of 18 mm). The extracts from the organically grown apple exhibited a clear antimicrobial activity, both against the strains of *B. cereus* and against the two strains of *E. coli*. The effectiveness found against the pathogenic microorganism *E. coli* DSM 8579 (an entero-haemorrhagic *E. coli*, of worldwide interest for the human health) in their study can be considered of promising importance as this strain produces verocytotoxins and causes haemorrhagic colitis and haemolytic uremic syndrome. Effect of water and alcohol extracts of *Malus domestica* (apple) was found to be effective against gram positive and gram negative bacteria- *B. subtilis, S. aureus, E. coli* and *P. aeruginosa* by Sun J. et al., [72].

The antibacterial effect of sweet lime on bacterial wound isolates was comparatively less than pomegranate, lemon and apple extracts. *P. aeruginosa* is the organism which is more inhibited (DIZ value of 12 mm) and remaining three bacteria *S. aureus*, *E. coli* and *K. pneumoniae* were equally inhibited with a DIZ value of 10 mm. In a similar study antimicrobial activities of *Citrus limetta* (sweet lime) were tested on 3 pathogenic bacteria- *P. aeruginosa*, *S. aureus* and *E. coli* by Mishra R. P. et al., [73]. A high antibacterial effect was recorded only against *P. aeruginosa* than the other two bacteria tested. These results are in accordance to results obtained in the present study wherein high antibacterial activity was observed for *P. aeruginosa*. In another study antibacterial effects of specific components of *Citrus limetta* on the growth of *Pseudomonas* species were analyzed by Oussalah M. et al., [74]. In their study limonene and –terpinene were isolated from sweet lime and their minimum inhibitory concentration (MIC) and maximal tolerated concentration (MTC) were estimated. Extracts of citrus fruits- lemon and lime showed good antibacterial activity against the pathogenic wound isolates. Lime juice has been locally applied on wounds and accidental cuts or abrasions in various Nigerian homes with the belief that this will speed up healing (Walker [75]).

In the present study tomato extracts exhibited least antibacterial activity than all five fruits tested against bacterial wound isolates with a DIZ values in range of 5 to 10 mm. High activity was observed against *S. aureus* and *E. coli* and least for *K. pneumoniae* (Table-4 and Figure-1). Similar studies on tomato were carried out by Du W. X. et al., [76], wherein tomato exerted more antibacterial effect on *E. coli* O-157:H7. The antibacterial action of extract obtained from tomato pulp has been studied by Vorob'ev A.A. et al., [77] with the use of museum strains *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 5638-P, *Candida albicans* ATCC 885-653, *Enterobacter, Streptococcus, Staphylococcus, Klebsiella* and *Escherichia* clinical strains. As revealed in this study, tomato pulp oil extract produces a wide-spectrum antibacterial effect on Gram-positive and Gram-negative microorganisms and on fungi of the genus Candida. The study has determined that this antibacterial action is linked with the presence of complex organic acids (succinic, citric, tartaric, etc.).

The results obtained for the fruit extracts in the present study showed significant antibacterial activity against the four bacterial wound isolates tested. Among all the tested bacteria *K. pneumoniae* is the least affected organism giving a clue that capsule and other determinant factors such as enzyme may be responsible for decreasing the effectiveness of the components present in fruit extracts. MIC by broth dilution showed good results compared to disc diffusion method as there may be a problem with the diffusion of the biological component into the agar. The hydrocarbon components either remain on the surface of the medium or evaporate (Griffin et al., [78]). That could be the reason for the better results obtained by the micro-dilution method. Broth method has advantage of lower workloads for a large number of replicates and the use of small volumes of the test substance and growth medium. (Sokovic et al., [79]). The difference in the antibacterial activity with the same source when extracted with different solvent has proven that not all phytochemicals that are responsible for antibacterial activity are soluble in a single solvent. Hence solvents of different polarity should be employed as discussed in this study (polar: water and nonpolar: ethanol).

CONCLUSION

In conclusion, the results obtained in this study clearly demonstrated broad spectrum antibacterial activity of Pomegranate (*Punica granatum*), Lemon (*Citrus limonium*), Apple (*Malus domestica*), and Sweet lime (*Citrus limetta*) against pathogenic bacteria of wound origin. Upon comparison with 8 commercial standard antibiotics, the antibacterial potentials of pomegranate ethanolic extracts on tested bacteria were better than 6 antibiotics: ampicillin, benzyl- penicillin, streptomycin, gentamycin, erythromycin and norfloxacin. Ethanolic extracts of lemon exerted better antibacterial effect than 4 antibiotics: ampicillin, benzyl-penicillin, streptomycin and norfloxacin. Apple ethanolic extracts exerted better than 3 antibiotics: ampicillin, benzyl-penicillin and norfloxacin and sweet lime better than 2 antibiotics: norfloxacin and benzyl-penicillin. The results suggest that these fruits contain active ingredients which qualify them for medicinal use. Importantly the results indicated that ethanolic extracts of these fruits are more effective than aqueous extracts. The presence of phytocompounds in the extracts including phenols, tannins and flavonoids as major constituents may be responsible for these antibacterial activities.

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