In *vitro* Antibacterial activity of *Phoenix loureiroi* KUNTH against selected Gram negative and Gram positive pathogenic bacteria

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Abstract*:*
In vitro antibacterial activity of *Phoenix loureiroi* fruit extract were examined using Ethanol, chloroform and aqueous as solvents were tested against human pathogen such as two grams Gram-positive: *Bacillus subtilis, Staphylococcus aureus* and five Gram-negative: *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhimurium, Serratia marcescens* by using a disc diffusion method. All the fruits extract shows significant activity against all human pathogens, but the Ethanol extract of *Phoenix loureiroi* showed maximum zone of inhibition against all the microorganisms than Chloroform. The minimum zones of inhibition were determined in water extract which shows less antibacterial activity against the pathogens strains. The result of an antimicrobial activity of three extract (Chloroform, Ethanol & Aqueous) of *Phoenix loureiroi* fruit showed positive effect in all tested microorganism. Thus it shows the efficacy of the plant *Phoenix a loureiroi fruit* has a possible source to obtain effective novel drugs to treat bacterial infections. Hence it is necessary for the tribal to uses of *Phoenix loureiroi* edible fruits as folk-fore medicine.

Keywords: Antibacterial activity, *Phoenix loureirii,* human pathogens, Disc diffusion method, novel drugs, folk-fore medicine.

1. Introduction:

Phoenix loureiroi is a common mountain dates palm. In Tamil, it is also called as siru Eecham. *Phoenix loureiroi* belongs to the family of Arecaceae. An arecaceae is one of the largest families in monocotyledons. It consists of 217 genera and more than 2,500 species. The members of this family are distributed throughout the tropical regions around the world. In India, it represented 25 genera and more than 225 species (Mandle *et al,* 2013). *Phoenix loureiroi* contains solitary and clustering plants with trunks from 1–4 m high and 25 cm in width, usually covered in old leaf bases. The leaves vary to some degree but usually reach 2 m in length with leaflets wide at the base and sharply pointed apices. The leaflets emerge from the rachis at varying angles creating a stiff, plumose leaf. The fruit is a single-seeded drupe, bluish-black

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when ripe, produced on erect, yellow inflorescences, usually hidden within the leaf crown. The species is noted for its variability in different habitats (Riffle *et al*, 2003).

Date fruits have a great significant for both a nutritional and therapeutic point of view (Fayadh, 1990; Besbes *et al*, 2004). They have sugars, vitamins, minerals and fibers in rich sources. In some varieties, the sugar content reaches up to 88%, and such fruits are considered a high-energy yield food (Al-Shahib and Marshall, 2003). Moreover, these fruits possess antioxidant and anticancer properties (Vayalil, 2002; Mansouri *et al*, 2002). It attributes high level polyphenolic compounds and also vitamins (Mansouri *et al*, 2002; Al-Turki, 2010). Dietary fibers are rich in dates variety for about 6.4%–11.5%, which improves their nutritive and therapeutic value (Al-Shahib and Marshall, 2003; Burt, 2004). Dates extracts have the antibacterial and antifungal properties (Sallal and Ashkenani, 1989; Shraideh, 1998; Selim *et al*, 1998). There is a great demand in treatment of various diseases such as arthritis, heart diseases and muscle aches and drug addiction. The microbes have an ability to develop resistant even in most powerful antimicrobial compounds (Abdul *et al*, 2012). Extraction of bioactive compound from medicinal plants leads to synthesis a more potent of new drug which reduced toxicity. Plant based extracts can be taken from any part of plant like barks, leaves, fruits, seeds and fruit rinds etc. (Abd Elgawad *et al*, 2015) The activity of various fruit extracts have been tested against various microorganisms like bacteria and fungi (Abd Elgawad *et al*, 2015) Many fruit have been used because of their antimicrobial traits due to the secondary metabolites compounds synthesized in the medicinal plant. This secondary metabolites compound is known by their active compound. For long time period, medicinally important fruit have been a valuable source for maintaining human health. The use of fruit and phytochemicals constituent has a both nutritive value and therapeutic treatments (Abd Elgawad *et al*, 2014). Recently there is no report submitted on the plant *Phoenix loureiroi* fruits extracts.

Therefore, this study focused on the fruit of *Phoenix loureiroi* which act against the bacterias such as *Bacillus subtilis*; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhimurium and Serratia marcescens.*

2. Materials and methods

2.1. Plant collection and identification:

Phoenix loureirii, Wild edible mature fruits were collected in the month of February to April at Kolli hills. The collected fruits specimen was authenticated by Botanical survey of India (BSI), Coimbatore, Tamil Nadu, India.

2.2. Preparation of Extract:

The samples were washed in running tap water and followed by sterile distilled water. These were dried in air room temperature for two days and Crushed to a fine powder using a sterilized mixer grinder and stored in airtight bottles. Three different solvents namely ethanol, Chloroform and aqueous were used for extraction. Ten grams of powdered fruits was separately soaked in 100ml of ethanol, Chloroform and sterile distilled water for 24 hours. Then the preparations were filtered through a Whatman No.1 filter paper and the filtered extract was concentrated under vacuum below 40^{o}C in rotary vacuum evaporator (Bag *et al*, 2009; Ogundiya *et al*, 2006). The

dried extract was exposed in UV rays for 24hrs for sterility and stored it in sterile labeled bottles in a freezer point at 4°C until for further use (Nkere and Iroegbu, 2005).

2.3. Tested organism:

Aqueous, Chloroform and Ethanol extract of crude drug was tested on various microorganisms such as two grams Gram-positive Bacterias: *Bacillus subtilis* MTCC 441; *Staphylococcus aureus* MTCC 740 and five Gram-negative Bacterias: *Escherichia coli* MTCC 2961; *Pseudomonas aeruginosa* MTCC 4676; *Klebsiella pneumoniae* MTCC 432; *Salmonella typhimurium* MTCC 733; *Serratia marcescens* MTCC 97 were procured from Microbial Type Culture Collection, Chandigarh. The microorganisms were subculture on the specific media such as Nutrient agar, Mueller-Hinton agar medium and also incubated at 37°C.

2.4. Antibacterial assay using Disc-Diffusion-Susceptibility method:

Antibacterial properties of the selected aqueous, chloroform and ethanol extracts were evaluated using Disc-diffusion-susceptibility method described by Bauer *et al*, (1966). The prepared Mueller-Hinton agar medium was poured into the pre-labeled sterilized petri plates. After solidification, freshly prepared culture in nutrient broth was swabbed with the help of sterilized cotton swab, carefully on the respective plates. Whatman filter paper discs (6mm diameter) impregnated with the test extracts were placed on the bacterial plates and solvent (aqueous, chloroform and ethanol) discs were used. Chloramphenicol (30 µg) standard antibiotics used as positive controls. Plates were incubated at 37^{o}C for 18-24 hours. All plates were used in triplicates and the average diameters of zone of inhibition on each plate were recorded.

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3. Result and Discussion:

In recent years, Multiple drug resistance microbes developed due to random use of commercial antimicrobial drugs commonly used for the treatment of various contagious diseases. This necessity situation leads to search for novel antimicrobial drugs and therefore the researchers gave an importance to herbal products, this leads to develop better drugs against microbial pathogen (Braga *et al*, 2005). The indeed emergence of an antibiotic resistance is further complicated by the bacterial resistant genes which travels faster and further most. Different sources needed to find out a new antimicrobial drug. Screening from plants material such as leaves, fruits, stem, bark, seed etc., is a validated method helps to find out potentially useful molecules against human pathogen diseases. (Karaalp *et al*, 2009). The secondary metabolites from the plant have an active compound which acts against the pathogen (Ushimaru *et al*, 2007).

In this present study, the antimicrobial activity was studied in ethanol, chloroform and aqueous fruit extract of *Phoenix loureiroi* and seven bacterias was selected such as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhimurium and Serratia marcescens.* The antimicrobial activity of the three extract at different concentrations (25µl, 50µl, 75µl, 100µl) were screened by the disc diffusion method and zones of inhibition were expressed in mean value. The antimicrobial

potential of aqueous, chloroform and ethanol extract of *Phoenix loureiroi* were showed in Tables 1-3 and graph1-3.

In aqueous extract, it shows the moderate growth inhibition of Gram positive and Gram negative bacteria. The bacteria's such as *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Serratia marcescens* shows the moderate zone of inhibition. *Klebsiella pneumonia, Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* shows very low zone of inhibition. In chloroform extract, the zonal formation was high in both Gram positive and Gram negative bacteria. The bacteria's such as *Bacillus subtilis* and *Staphylococcus aureus* shows very high activity. In ethanol extract, high inhibition against the growth of Gram positive and Gram negative bacterias. The bacteria's such as Gram-positive: *Bacillus subtilis*; *Staphylococcus aureus* and five Gram-negative: *Escherichia coli*; *Pseudomonas aeruginosa*; *Klebsiella pneumoniae*; *Salmonella typhimurium*; *Serratia marcescen has* very high zonal formation that shows the high inhibitory against this pathogen. The results indicated that the extracts showed antibacterial activities towards the Gram-positive and Gram-negative bacteria, but with variability related to the bacterial genus and species. Gram-negative bacteria were more susceptible than Gram-positive bacteria**.** The difference of Gram-positive and Gram-negative bacterias is due to structural changes in cell wall of those bacteria's. The Gram-negative cell wall is complex and multilayered structure; it has an outer phospholipids membrane carrying the structural lipopolysaccharide components, which makes a barrier to many environmental substances including synthetic and natural antibiotics. The Gram-positive bacteria contain a single outer peptidoglycan layer, which is not an effective permeability barrier (Sexena *et al,* 2004). Sadaf Zehra and his coworker (2015) reported that the palm fruit such as *Phoenix dactylifera* extracts did not exhibit any antibacterial activity. Other researchers have found that the small sucrose derivatives could not inhibit the growth of *E. coli*. The resistance is attributed to the cytoderm lipopolysaccharide and membrane lipids which could screen out the fatty acid and prevent transport in the cell membrane (Ferrer *et al,* 2005). Other report also support the resistance of gram negative bacteria to the inhibitory effects of the sugar esters because of membrane structure and difference in cell wall (Ouattara *et al,* 1997). But the fruits of the *Phoenix loureiroi* show the best result in inhibiting the growth of bacterias. On the other hand, chloramphenicol as positive control, showed toxicity against all seven examined pathogenic strains. The activity of mountain date palm *Phoenix loureiroi* extracts as antibacterial agent may be due to the ability of phenolic compounds to bind with the bacterial cell wall and therefore inhibiting the bacterial growth (Barbary *et al*, 2010). Polyphenols played an important role in protein precipitation and enzyme inhibition of microorganisms (Naz *et al,* 2007). The results showed better due to phenolic compounds and antimicrobial activity. The result shows the confirmed potentiality of mountain date palm *Phoenix loureiroi* fruits act as antibacterial agent as well as good dietary food. This study on antibacterial potential of *Phoenix loureiroi* proved that the ethanol as a better solvent for extraction of antimicrobial agents than chloroform and aqueous because the ethanol has an to solubilize and extract some active compounds such as flavonoids, phenol, tannin etc., which responsible for antimicrobial activity. These active compounds have been an effective

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source of inhibiting various microorganisms (Rashid *et al*, 2013). Moreover, ethanol formulations are relatively safe for human consumption as compared with other organic solvents such as chloroform ethyl acetate, methanol etc. This might be the reason that ethanol extracts showed better antimicrobial activity against test organisms (Wendakoon *et al*, 2012).

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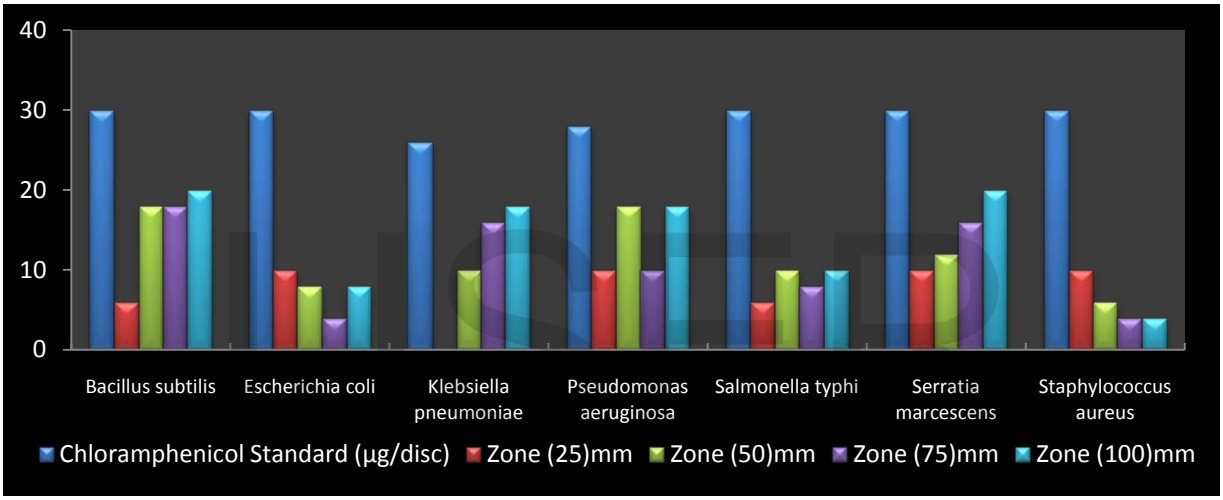
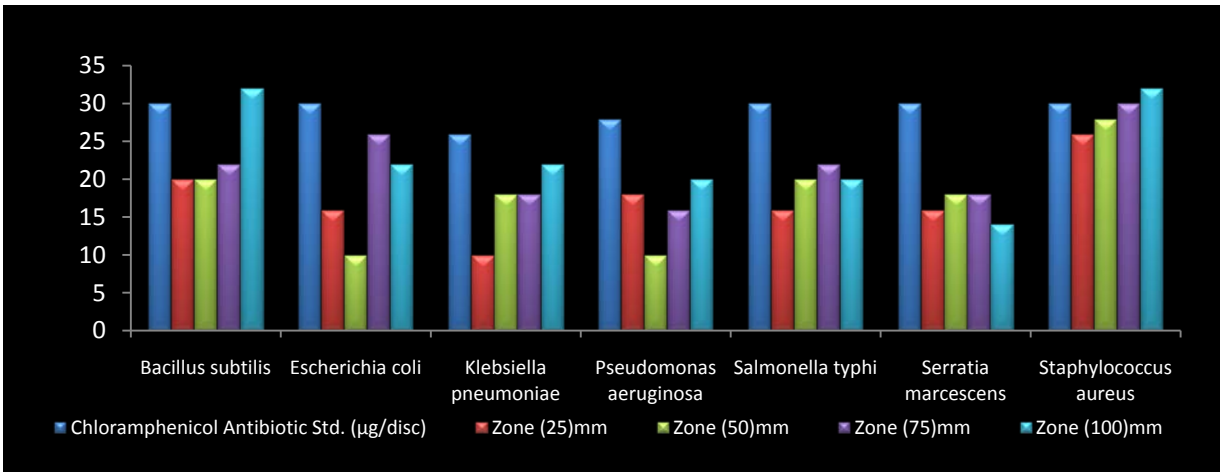
Table-1 Antibacterial activity of Aqueous extract of *Phoenix loureiroi* fruits						
S.No.	**Microorganisms**	**Chloramphenicol (µg/disc)**	**Zone of inhibition (mm)**			
			25(µg/ml)	**50**(µg/ml)	**75**(µg/ml)	**100**(µg/ml)
1.	*Bacillus subtilis*	30	6±0.01	18±0.02	18±0.1	20±0.002
2.	*Escherichia coli*	30	10±0.1	8±0.04	4±0.21	8±0.12
3.	*Klebsiella pneumoniae*	26	0±0.04	10±0.16	16±0.001	18±0.1
4.	*Pseudomonas aeruginosa*	28	10±0.14	18±0.02	10±0.52	18±0.26
5.	*Salmonella typhi*	28	6±0.012	10±0.1	8±0.32	10±0.18
6.	*Serratia marcescens*	30	10±0.02	12±0.02	16±0.04	20±0.006
7.	*Staphylococcus aureus*	30	10±1.01	6±0.01	4±1.1	4±1.2



Graph -1 Antibacterial activity of aqueous extract of *Phoenix loureiroi* fruits

Table-2 Antibacterial activity of Chloroform extract of *Phoenix loureiroi* fruits						
S.No	**Microorganisms**	**Chloramphenicol (µg/disc)**	**zone of inhibition (mm)**			
			25(µg/ml)	**50**(µg/ml)	**75**(µg/ml)	**100**(µg/ml)
1.	*Bacillus subtilis*	30	16±0.02	16±0.12	18±0.41	32±0.02
2.	*Escherichia coli*	30	16±0.1	10±0.02	26±0.04	22±0.42
3.	*Klebsiella pneumoniae*	26	10±0.01	18±0.02	18±0.3	22±1.02
4.	*Pseudomonas aeruginosa*	28	18±0.02	10±0.1	16±0.48	20±0.1
5.	*Salmonella typhi*	28	16±0.22	20±0.02	22±0.02	20±0.02
6.	*Serratia marcescens*	30	16±1.2	18±0.4	18±0.1	14±1.0
7.	*Staphylococcus aureus*	30	26±0.04	28±0.14	30±0.21	32±0.01



Graph -2 Antibacterial activity of chloroform extract of *Phoenix loureiroi* fruits

Table-3 Antibacterial activity of Ethanol extract of *Phoenix loureiroi* fruits

S.No.	Microorganisms	Chloramphenicol (µg/disc)	zone of inhibition (mm)			
			25(µg/ml)	**50**(µg/ml)	**75**(µg/ml)	**100**(µg/ml)
1.	*Bacillus subtilis*	30	28±0.02	24±0.02	14±0.06	32±0.001
2.	*Escherichia coli*	30	18±0.01	20±0.01	22±0.04	30±0.02
3.	*Klebsiella pneumoniae*	26	16±1.4	16±0.04	10±1.2	26±0.02
4.	*Pseudomonas aeruginosa*	28	18±1.02	20±0.01	18±1.0	28±0.1
5.	*Salmonella typhi*	28	18±0.2	24±0.12	20±0.01	28±1.04
6.	*Serratia marcescens*	30	20±1.2	26±1.02	22±0.01	32±0.004
7.	*Staphylococcus aureus*	30	04±0.06	28±0.1	10±0.02	18±1.52

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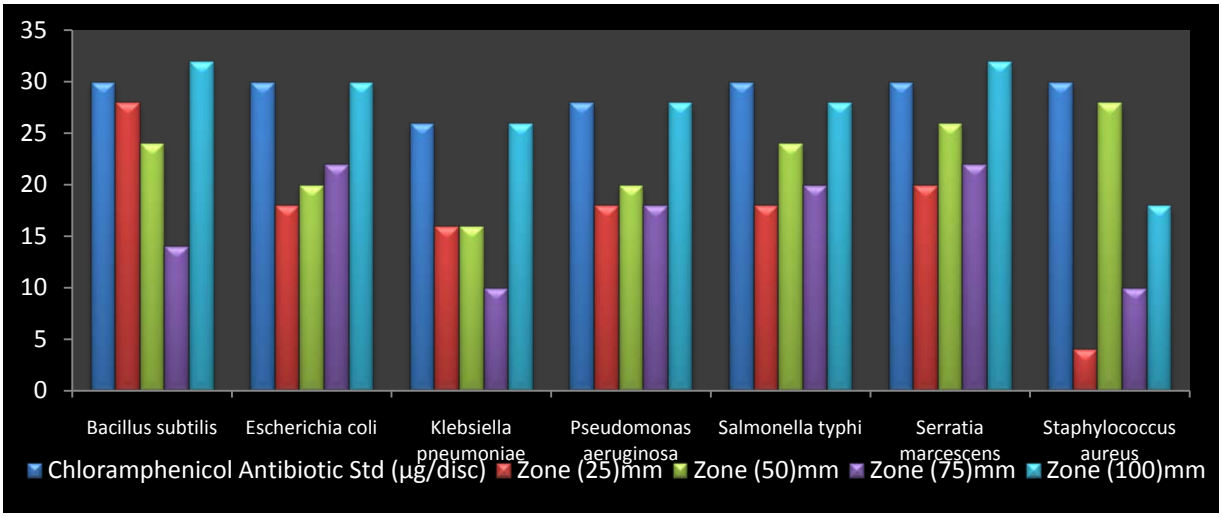

Graph -3 Antibacterial activity of ethanol extract of *Phoenix loureiroi* fruits

CONCLUSION

Phoenix loureiroi fruit is a wild edible has a traditional medicinal value and nutritive value in the tribal resident study area. Thus the *Phoenix loureiroi* fruits is taken for the research work, it is concluded that *Phoenix loureiroi* fruit has beneficial effect against tested pathogens. Three different solvent of the extract has an active compound which inhibits the growth of *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhimurium* and *Serratia marcescens*. This study reveals the potential use of these fruits for developing new antimicrobial drug against pathogenic micro organisms. These findings may helps to discover new chemical classes of antibiotic compound that could serve as novel drug for diseases cause pathogen.

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