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Comparative study of different solvent extract of *Olea dioica* Roxb. Western Ghats, Karnataka, against selected plant and animal pathogenic bacteria

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A B S T R A C T

Olea dioica Roxb. is a very important ethno medicinal evergreen tree grown abundantly in Western Ghats, India. This plant parts used by tribal people in sidda treatment. Studies were conducted on the anti-microbial properties of compound extracted from *Olea dioica* Roxb. Which was collected from selected parts of the Western ghats, Karnataka in April 2014. The present study was conducted to study the effectiveness of the plant crude extract against some animal and plant pathogenic microbes. Antimicrobial activity was assessed by agar well diffusion method against nine bacterial strains. The crude extracts (pet.ether, chloroform and methanol crude extracts) tested against 9 bacterial strains (3 plant pathogenic and 6 animal pathogenic). The results showed that the only methanoliccrude extract of *Olea dioica* Roxb. was effective against the all the microbes with fewer effect on the microbes by other two crude extracts (Petroleum ether and chloroform).

Keywords: Solid Dispersion, Skimmed milk powder, solubility, Pioglitazone, Dissolution

A R T I C L E I N F O

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1. Introduction

Herbal drugs have been used since ancient times as medicines for the treatment of range of diseases. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Therefore, several medicinal plants have been evaluated for possible antimicrobial activity and to get remedy from a variety of ailments of microbial origin (Subramani and Goraya, 2003). Secondary metabolites like flavonoids (Ruddock PS et al., 2011), terpenoids (Singh and Singh 2003), steroids (Taleb-Contini SH et al., 2003), saponins (Mandal P et al., 2005), glycosides (Nazemiyeh H et al 2008) which are extracted from higher plants has confirmed the antimicrobial properties.

Olea dioica Roxb. is a tree species belongs to family of Oleaceae grows in evergreen to semi-evergreen and moist deciduous type up to 1200maltitude forests of Western ghat, India. Trees up to 15 m tall. Bark brownish, rough; blaze pale brown. The roots of the plant used for cancer and snake bite treatment in siddha medicine having good ethno botanic medicinal properties and bark, fruit paste is used in the treatment of rheumatism; decoction of the bark is used to wash old wounds and given in fever. In Maharashtra, the tribes uses *Olea dioica* Roxb. fruits in the treatment of various skin diseases. (Pullaiah T. Biodiversity in India. 2006). Ripe fruits are traditionally used in the various treatment, by the tribes in Kerala forest (Yesodharan K and Sujana KA 2007).

2. Experimental

Plant material

The leaf of *Olea dioica* Roxb. Collected from Narasimha Parvata in Kigga, Shringeri taluk, Karnataka in April 2014.The botanical identification of the plant was done by Prof. K G Bhat, Udupi and the voucher specimen was conserved under the reference number KU/AB/RN/001.

Purification and general procedures

The leaf samples were shade dried for about 20 to 25 days and mechanically powdered. The powdered material was subjected to Soxhlet extraction by with petroleum ether, chloroform, and methanol successively. The crude extracts were concentrated to dryness in a rotary flash evaporator under reduced pressure and controlled temperature. Stored in cool condition in air tight plastic containers.

Culture media and bacterial strains:

For culture of test organisms NA (nutrient agar) media is used and the all the 9 bacterial strains obtained from Institution of Microbial Technology (IMTECH), Chandigarh, India. *Xanthomonas campestris* (MTCC-2286), *Pseudomonas syringae* (MTCC-1604), *Agrobacteirum tumefaciens* (MTCC-431), *Klebsiella pneumonia* (MTCC-7028), *Escherichia coli* (MTCC 1559), *Salmonella typhi* (MTCC-734), *Pseudomonas aeruginosa* (MTCC-1934), *Staphylococcus aureus* (MTCC-902). *Streptomyces pneumonieae* (MTCC-4734) were used against the crude extracts of Olea dioica Roxb.

Anti-bacterial assay: Antibacterial activity was performed through agar well diffusion method by standard protocol (Murray PR et al 1995). The test bacteria were aseptically inoculated in sterile test tubes using nutrient broth and incubated at 37^0C for 24 hours.

The plant crude extracts were dissolved in 10% DMSO to get desired concentrations of 12.5, 25, 50 and 100 mg/ml respectively. The drug Ciproflaxin was used as standard antibiotic (1mg/ml of sterile distilled water) to compare with the plant crude extracts. Nutrient agar plates were prepared and the broth cultures of bacterial strains were uniformly swabbed. 0.6 cm diameter wells were punched in the inoculated plates using a sterile cork borer.100 µl of different concentrations of crude extracts and standard (Ciproflaxin, 1mg/ml of sterile distilled water) and DMSO (10%) were filled into the respectively labeled wells and incubate for 24 hours at 37^oC.

3. Results and Discussion

The petroleum ether crude extract of *Olea dioica* shows fewer antimicrobial properties in 100 mg/ml concentration shows 6 mm zone for *Staphylococcus aureus* and 6 mm for *Escherichia coli*, but for other microorganisms the petroleum ether crude extract shows no effect. (Table 1). The chloroform crude extract of *Olea dioica* shows better antimicrobial properties than the petroleum ether.

The extract in 100 mg/ml concentration shows 6 mm zone for *Staphylococcus aureus*, 6 mm *for Salmonella typhi*, 6 mm Pseudomonas syringae, 7 mm for Pseudomonas aeruginosa, 7 mm Xanthomonas campestris, 6 mm Escherichia coli, 7 mm Streptomyces pneumonia, butit shows no effect on Klebsiella pneumonia and *Agrobacterium tumefaciens.* (Table 2). The methanolic crude extract of *Olea dioica* shows best antibacterial properties than the rest of two crude extracts.

The methanolic crude extract form 12.5 mg/ml concentration to 100 mg/ml shows effective inhibitory zones against all test bacterial strains, in that *Escherichia coli* more susceptible to the extract with the highest zone of inhibition of 7 mm in 12.5mg/ml concentration of extract but the *Agrobacterium tumefaciens*and *Pseudomonas syringae* show no zone of inhibition in 12.5 mg/ml concentration. In 25 mg/ml concentration of extract showed maximum inhibitory activity for *Escherichia coli* 9 mm followed by *Pseudomonas aeruginosa*8 mm and in 25 mg/ml concentration of methanolic crude extract all the test organism shows susceptibility.

In 50 mg/ml concentration of methanolic extract shows the zone of inhibition against all the organisms highest is *Escherichia coli* with13.6 mm zone followed by *Staphylococcus aureus* with 13.6 mm zone. In 100 mg/ml concentration of crude extract highest *Escherichia coli* with20.3 mm followed by *Salmonella typhi* with 19.6 mm zone. (Table 3). The standard drug ciprofloxin shows effect on all the test organisms with highest 40mm zone and lowest of 34.3 mm zone and the control DMSO shows nil effect on the test pathogenic bacteria.

Table 1: Pet.ether crude extracts of *Olea dioica* Roxb.against test bacterial strains

Test organisms	Zone of inhibition in mm (Mean±SD)				Standard (Ciproflaxin)
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	
Sa	0±0	0±0	0±0	6±0.05	39±0.0.05
St	0±0	0±0	0±0	0±0	36±0.1
Kp	0±0	0±0	0±0	0±0	35±0.11
At	0±0	0±0	0±0	0±0	33±0.11
Ps	0±0	0±0	0±0	0±0	34±0.057
Pa	0±0	0±0	0±0	0±0	41±0.11
Xc	0±0	0±0	0±0	0±0	35±0.1
Ec	0±0	0±0	0±0	6±0	35±0.05
Sp	0±0	0±0	0±0	0±0	34±0.05

Sa: Staphylococcus aureus,St: Salmonella typhi, Kp:Klebsiella pneumonia, At:Agrobacterium tumefaciens, Ps:Pseudomonas syringae, Pa:Pseudomonas aeruginosa, Xa:Xanthomonas campestris, Ec: Escherichia coli, Sp: Streptomyces pneumonia.

Table 2: Chloroform crude extract of *Olea dioica* Roxb.against test bacterial strains

Test organisms	Zone of inhibition in mm (Mean ± SD)				Standard (Ciproflaxin)
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	
Sa	0±0	0±0	0±0	0.6±0	35±0.05
St	0±0	0±0	0±0	0.6±0	35±0.1
Kp	0±0	0±0	0±0	0±0	39±0.1
At	0±0	0±0	0±0	0±0	34±0.05
Ps	0±0	0±0	0±0	6±0.05	35±0.05
Pa	0±0	0±0	0±0	7±0.05	35±0.1
Xa	0±0	0±0	0±0	7±0	32±0.11
Ec	0±0	0±0	0±0	6±0.05	35±0.1
Sp	0±0	0±0	0±0	7±0.05	40±0.11

Sa: Staphylococcus aureus, St: Salmonella typhi, Kp:Klebsiella pneumonia, At: Agrobacterium tumefaciens, Ps: Pseudomonas syringae, Pa: Pseudomonas aeruginosa, Xa:Xanthomonas campestris, Ec: Escherichia coli, Sp: Streptomyces pneumonia.

Table 3: Methanol crude extract of *Olea dioica* Roxb.against test bacterial strains

Test organisms	Zone of inhibition in mm (Mean±SD)				Standard (Ciproflaxin)
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	
Sa	7±0.05	12±0.05	13±0.05	18±0.05	39±0.1
St	6±0	8±0.05	11±0.05	19±0.05	35±0.05
Kp	6±0	10±0.05	12±0	18±0.05	35±0.1
At	0±0	6±0.05	8±0.11	11±0.11	32±0.11
Ps	0±0	6±0	7±0.05	10±0	35±0.1
Pa	6±0.05	8±0.05	12±0	17±0.05	40±0.11
Xc	7±0.05	8±0.05	12±0.05	17±0.11	34±0.05
Ec	7±1.3	9±0.11	13±0.05	20±0.05	35±0.05
Sp	6±0.05	10±0.05	1.2±0	17±0.05	35±0.1

Sa: Staphylococcus aureus, St: Salmonella typhi, Kp: Klebsiella pneumonia, At: Agrobacterium tumefaciens, Ps: Pseudomonas syringae, Pa: Pseudomonas aeruginosa, Xa:Xanthomonas campestris, Ec: Escherichia coli, Sp: Streptomyces pneumonia.

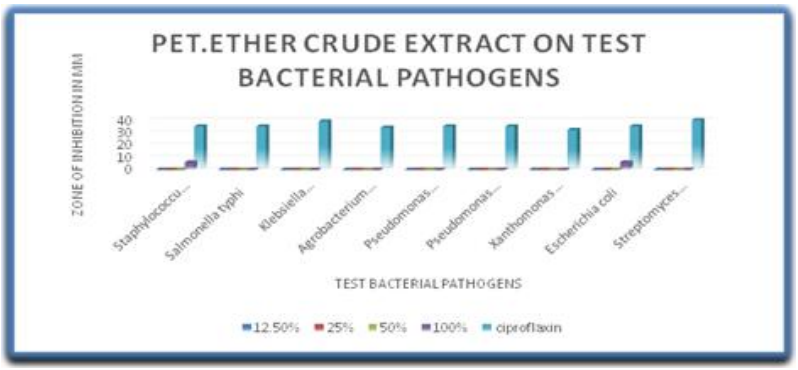

Chart 1: Pet.ether crude extract on test bacterial pathogens

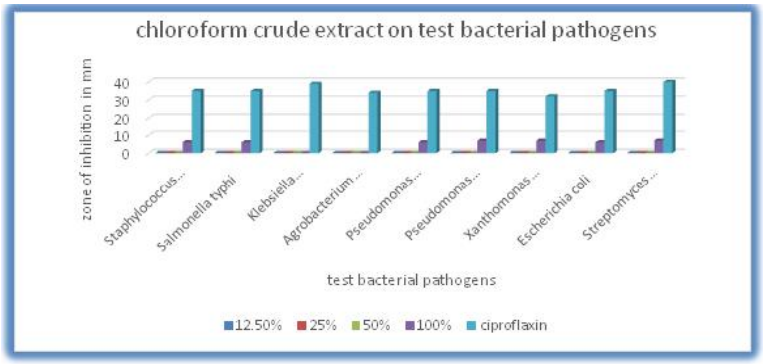

Chart 2: Chloroform crude extract on test bacterial pathogens

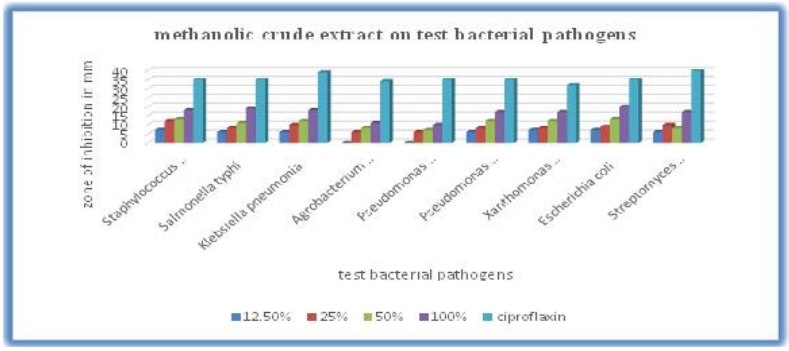

Chart 3: Methanolic crude extract on test bacterial pathogens

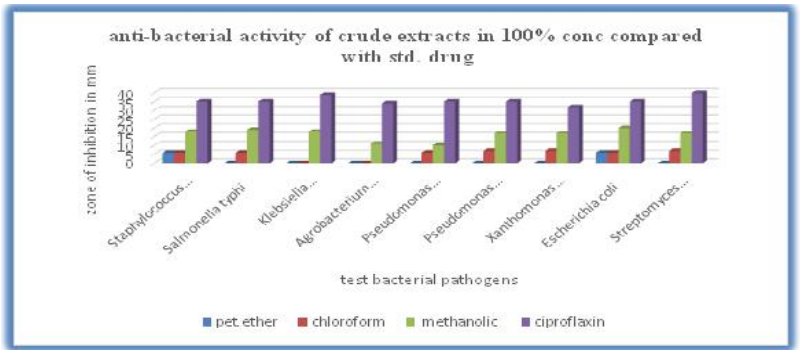

Chart 4: Anti-bacterial activity of crude extracts in 100% conc. compared with std. drug

Discussion

The infection caused by the pathogens is a complex reaction with the host organism. After discovery and use of many drugs and antibiotics there is a report that the pathogens are gradually gaining the resistance to the used drugs such as Fluconazole resistant *C.albicans*, Vancomycin and Methicillin resistant *S.aureus*, *Enterococci*, Multidrug resistant TB etc. these reasons are alarming the scientist to discover new drugs search from other sources, particularly from plants. Plants are the rich sources of unknown chemicals which will be used as drugs to cure many disorders. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982). It has been recorded that 80% of the world's population has fidelity in traditional medicine, particularly plant based drugs for their primary healthcare (Valdiani et al., 2011).

The experiment revealed that only the methanolic crude extract of *Olea dioica* Roxb. effective against plant and animal pathogenic bacterial strains with the negligible anti-bacterial activity of other two crude extracts (pet.ether and chloroform crude extracts), and it also revealed that the methanolic crude extract is more effective against animal pathogen than the plant pathogens with the highest zone of inhibition for *Escherichia coli* (20.3±0.05 mm), *Salmonella typhi* (19.6±0.05), *Staphylococcus aureus* (18.6±0.05), *Klebsiella pneumonia* (18.3±0.05) in 100 mg/ml concentration. The plant pathogens are less susceptible to the methanolic crude extract of *Olea dioica* Roxb. Plant with the minimal inhibitory zone viz., *Pseudomonas syringae*(10±0),*Agrobacterium tumefaciens*(11.3±0.11), *Xanthomonas campestris*(17.3±0.11) in 100 mg/ml concentration. In 25 mg/ml and 50 mg/ml concentration of methanolic crude extract is effective for all tested pathogens with the highest zone of inhibition for *Staphylococcus aureus* (12±0.05) and *Salmonella typhi* (19.6±0.05) in the

respective concentrations. In 12.5 mg/ml concentration of methanolic crude extract was nil effect on *Agrobacterium tumefaciens* (0±0), *Pseudomonas syringae* (0±0) pathogens and highest effect on *Staphylococcus aureus* (7.3±0.057) *and Xanthomonas campestris* (7.3±0.05). The methanolic crude extract shows maximum inhibition zone for *Escherichia coli*, *Staphylococcus aureus* which causes food infections and *Salmonella typhi* which causes typhoid infection shows that the plant methanolic crude extract might be useful in the treatment of these bacterial infections. Tannins and saponnins are reported to have antimicrobial activity (Evans W.C. (1999)). These antibacterial property may due the presence of flavonoids, glycosides, phenols, alkaloids, saponins and sterols etc. In our preliminary phytochemical tests we got the positive results for saponins, flavonoids, steroids/sterols, glycosides, phenols which is the reason for the antibacterial properties of the plant methanolic crude extract and control DMSO shows nil effect on the pathogen which will in turn shows the positive activity of the methanolic crude extract.

4. Conclusion

Our work concluded that only methanolic crude extract of *Olea dioica* Roxb. shows appreciable antibacterial activities against test pathogen and especially the methanolic crude extract is more effective against animal pathogenic bacteria than the plant pathogenic bacteria. The antibacterial properties due the presence of various secondary metabolites such as saponins, flavonoids, steroids/sterols, glycosides, phenols which is also confirmed by respected confirmation tests. Further studies on specific compound may reveals the activity in particular.

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