Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(4): 156-160
Received: 05-09-2015
Accepted: 07-10-2015

Ashwathanarayana R
Research student, Department of PG Studies and Research in Applied Botany, Jnanasahyadri, Kuvempu University, Shankaraghatta- 577451. Shimoga, district, Karnataka, India.

Raja Naika
Professor, Department of PG Studies and Research in Applied Botany, Jnanasahyadri, Kuvempu University, Shankaraghatta- 577451. Shimoga, district, Karnataka, India.

Correspondence:
Ashwathanarayana R
Research student, Department of PG Studies and Research in Applied Botany, Jnanasahyadri, Kuvempu University, Shankaraghatta- 577451. Shimoga, district, Karnataka, India.

Preliminary phytochemical and antimicrobial properties of *Olea dioica* Roxb bark extract collected from Western Ghats, Karnataka, India

Ashwathanarayana R, Raja Naika

Abstract
Olea dioica Roxb.(F.: Oleaceae), an important ethno-medicinal tree, grows in open, evergreen to semi-evergreen and moist deciduous forests up to 1200 m and is distributed throughout the Western Ghats, India. The plant parts such as roots and leaves are used in siddha medicine to cure cancer and for treatment of snake bite. For preliminary phytochemical analysis and antimicrobial assay, bark samples were collected, shade-dried for 21 days and subjected for soxhlet extraction. Polarity wise the solvents used to extract the crude compound are, petroleum ether, chloroform and methanol. In the preliminary phytochemical analysis, petroleum ether showed negative results and chloroform crude extract shows positive result for Tannins and flavonoids. The methanolic crude extract showed positive reaction for Saponins, Flavonoids, Steroids, Glycosides, Phenols and Sterols. The bark crude extract of *Olea dioica*,. was screened against four pathogenic fungal strains and nine pathogenic bacterial strains by zone inhibition test. For fungal pathogens all extract shows nil effect so that the bark crude extract was no effect on tested fungal pathogens. For bacterial strains the crude extract shows dose dependent inhibition. The maximum inhibition zone observed in methanolic crude extract for *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia,* but for pathogenic fungal strains it showed nil effect. The petroleum ether and chloroform shows negligible antimicrobial activity against almost all tested microbial strains and it also showed that the methanolic crude extract effective against test bacterial strains but not effective against pathogenic fungal strains.

Keywords: *Olea dioica* Roxb, Western Ghats, Karnataka, Preliminary Phytochemical analysis, antimicrobial activity.

1. Introduction
Medicinal plants have been used for centuries as remedy for human diseases. These plants are sources of biologically active chemical compounds and some of them are anti-microbial agents [1]. Several medicinal plants have been evaluated for possible antimicrobial activity and to get remedy for a variety of ailments of microbial origin [2-5]. Secondary metabolites such as flavonoids [6], terpenoids [8], steroids [9], saponins [9], glycosides [10] extracted from higher plants have antimicrobial properties.

Olea dioica Roxb. Is an important ethno-medicinal tree belonging to the family Oleaceae. The tree grows up to 15 m tall. Bark of the tree is brownish, rough; blaze pale brown. Young branchlets are subquadrangular, lenticellate, glabrous. Leaves are simple, opposite, decussate; petiole 0.6-1.3 cm long, canaliculate; lamina 7.5-17.5 x 2.3-7.5 cm, elliptic to elliptic-oblong, apex gradually acuminate to subacute, base acute or attenuate, margin distantly serrate (with strong teeth) or entire, coriaceous to subcoriaceous, glabrous; midrib flat above, usually reddish when dry; secondary nerves 8-12 pairs; tertiary and higher order nerves obscure or slightly impressed. Inflorescence axillary divaricate panicles; flowers polygamodioecious, cream-white; pedicel 0.4 cm long. Fruit is drupe, ellipsoid, blue when ripe; one-seeded. Roots of the plant have medicinal properties and are used for treatment of cancer and snake bite in siddha medicine. In Maharashtra, the tribes use *Olea dioica* Roxb. Fruits for treatment of skin disease. Bark and fruit paste are used in rheumatism; decoction of the bark is used to wash old wounds and given to counter fever [11]. Ripe fruits are traditionally used by the tribes in Kerala forest [12]. *Olea dioica* leaf methanolic extract showed appreciable antibacterial and antifungal activity [13, 14].

Materials and Methods

Plant collection and authentication

The bark material of *Olea dioica* Roxb. Were collected from Narasimha Parvata in Kigga, Shringeri taluk, Karnataka in April 2014. The plant was identified by Prof. K G Bhat, Udupi and a voucher specimen was conserved under the reference number KU/AB/RN/AS/001.

Plant preparation and extraction

The bark samples were dried in shade for 20 to 25 days, mechanically powdered and subjected to Soxhlet extraction using petroleum ether, chloroform, and methanol [15]. the crude extracts were collected in air-tight plastic containers and stored in cool condition.

Preliminary phytochemical screening

Air-dried and powdered bark materials and also all crude extracts were screened for the presence of tannins, alkaloids, saponin, glycosides, flavonoids, steroids/sterols and phenols using standard methods [16-18].

Microorganisms used

Pathogenic fungal strains like *Candida albicans*, *Chrysosporium merdarium*, *Trichophyton rubrum* and *Chrysosporium keratinophilum* and pathogenic bacterial strains like *Xanthomonas campestris* (MTCC-2286), *Pseudomonas syringae* (MTCC-1604), *Agrobacterium tumefaciens* (MTCC-431), *Klebsiella pneumonia* (MTCC-7028), *Escherichia coli* (MTCC 1559), *Salmonella typhi* (MTCC-734), *Pseudomonas aeruginosa* (MTCC-1934), *Staphylococcus aureus* (MTCC-902). *Streptomyces pneumoniae* (MTCC-4734) obtained from the Institution of Microbial Technology (IMTECH), Chandigarh, India were used.

Medium used

Nutrient agar (NA) media for bacterial pathogens and Sabouraud dextrose agar (SDA) used for the culturing of experimental fungal pathogens.

Standard drug

Antifungal drug Fluconazole (1mg/ml of sterile distilled water) and anti-bacterial drug Ciprofloxacin were (1mg/ml of sterile distilled water) used as standard antibiotic to compare with the plant crude extracts.

Preparation of microbial cultures

For fungi

The test fungi were aseptically inoculated in sterile test tubes using Sabouraud dextrose broth and incubated at 28 ^{0}C for 36-48 h. The plant crude extracts were dissolved in 10% DMSO to get desired concentrations of 12.5, 25, 50 and 100 mg/ml respectively. Sabouraud dextrose agar (SDA) plates were prepared and the broth cultures of fungal strains were uniformly swabbed with the help of tween-20. 6 mm diam wells were punched in the inoculated plates using a sterile cork borer. One hundred µl of different concentrations of extract and standard (Fluconazole, 1mg/ml of sterile distilled water) and DMSO (10%) were filled into the respectively labeled wells and incubated for 36-48 h at 28 ^{o}C.

For bacteria

The test bacteria were aseptically inoculated in sterile test tubes using nutrient broth and incubated at 370C 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 Ciprofloxacin 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 (Ciprofloxacin, 1mg/ml of sterile distilled water) and DMSO (10%) were filled into the respectively labeled wells and incubate for 24 hours at 37 ^{o}C.

Results

Preliminary phytochemical screening

The soxhlet extraction of *Olea dioica* bark (750 g) with petroleum ether gives 20.34 g, with chloroform gives 18.64 g and with methanol gives 35.32 g yield (Chart 1). The results of phytochemical screening of *Olea dioica* bark indicate the presence of Saponins, Flavanoids, Steroids/Sterols, Glycosides and Phenols in methanol crude extract, the chloroform crude extracts shows positive results for Tannins and Flavonoids but the petroleum ether crude extract gave negative results for all these compounds (Table 1).

Anti-microbial activity of different extract

Petroleum ether crude extract against test pathogens

Petroleum ether crude extract show nil effect on all tested microorganisms (Table 2) which indicates that petroleum ether crude extract is not effective against tested pathogens.

Chloroform crude extract against test pathogens

Chloroform crude extract show negligible effect on all tested microorganisms (Table 3). In 100% concentration it shows negligible effect on the bacterial strains except *Agrobacterium tumefaciens.* Chloroform crude extract shows nil effect on the all the tested fungal strains in all concentrations.

Methanolic crude extract against test pathogens

The methanolic crude extract shows appreciable antibacterial activity against all tested pathogens in 25, 50 and 100% concentrations but it doesn't shows antifungal activity in all concentrations (Table 4). The methanolic extract shows highest zone of inhibition against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumonia*. (Table 4).

In 12.5 % only two bacterial strains viz., *Klebsiella pneumonia* and *Streptomyces pneumonia* were susceptible for crude methanolic extract and successively in 25, 50 and 100% concentrations all the bacterial organisms are susceptible to the methanolic crude extract. (Chart 2)

From the study, it revealed that *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumonia* were more susceptible to the methanolic crude extract and *Xanthomonas campestris*, *Pseudomonas aeruginosa*, *Streptomyces pneumonia*, *Pseudomonas syringae* and *Agrobacterium tumefaciens*, were moderately susceptible

to methanolic extract. In the 100% concentration the methanolic crude extract shows appreciable antibacterial activity.

This also reconfirms the positive activity of methanolic crude extract of *Olea dioica* against *Xanthomonas campestris*, *Escherichia coli*, *Staphylococcus aureus* which Prashith [17] previously worked out for leaf extract but, during our study we examined more pathogens with different parts (bark, root) of the plant.

Standard drugs fluconazole and Ciprofloxacin were showed zone for all tested pathogens. Control DMSO did not show any zone of inhibition and it in turn confirms the positive activity of methanolic crude extract. In present days pathogens were getting resistance against the frequently used drugs due to this invention of new moderated drugs were necessary. From our results it is concluded that the methanolic bark crude extract shows appreciable antibacterial activity against tested pathogens it might be useful in the treatment of those infections. These antibacterial properties may due the presence of flavonoids, glycosides, phenols, alkaloids, saponins and sterols (Table 1).

Table 1: Preliminary phytochemical Analysis

Secondary Metabolites	Type of tests	Petroleum ether crude extract	Chloroform crude extract	Methanol crude extract
Alkaloids	Mayer's test	-	-	-
	Wagner's test	-	-	-
Saponins	Foam test	-	-	+
Tannins	Ferric chloride test	-	+	+
Flavonoids	Shinda test	-	-	+
	Zinc -HCl reduction test	-	-	+
	Alkaline reagent test	-	-	+
	Lead acetate test	-	+	+
Steroids	Salkowaski test	-	-	+
Glycosides	Keller-Killianis test	-	-	+
	Brown water test	-	-	+
	Legal test	-	-	+
Phenols	Ferric chloride test	-	-	+
	Acetic acid test	-	-	+
Sterols	Liebermann burchad test	-	-	+

Negative result, +: positive result.

Table 2: Petroleum ether crude extract of *Olea dioica* against test microbial strains

Test organisms	Zone of inhibition in mm (Mean±SD)				
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	
Fungalstrains					**Standard (fluconazole)**
Ca	0±0	0±0	0±0	0±0	30±0.05
Cm	0±0	0±0	0±0	0±0	40±0
Tr	0±0	0±0	0±0	0±0	43±0.05
Ck	0±0	0±0	0±0	0±0	45±0
Bacterial strains					**Standard (Ciprofloxacin)**
Sa	0±0	0±0	0±0	0±0	34±0
St	0±0	0±0	0±0	0±0	35±0.11
Kp	0±0	0±0	0±0	0±0	37±0.05
At	0±0	0±0	0±0	0±0	35±0.05
Ps	0±0	0±0	0±0	0±0	32±0.05
Pa	0±0	0±0	0±0	0±0	34±0.05
Xc	0±0	0±0	0±0	0±0	31±0.05
Ec	0±0	0±0	0±0	0±0	34±0.05
Sp	0±0	0±0	0±0	0±0	36±0.05

Ca: Candida albicans, Cm: Chrysosporium merdarium, Tr: Trichophyton rubrum, Ck: Chrysosporium keratinophilum, 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: Chloroform crude extract of *Olea dioica* against test microbial strains

Test organisms	Zone of inhibition in mm (Mean±SD)				
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	
Fungal strains					**Standard (fluconazole)**
Ca	0±0	0±0	0±0	0±0	30±0.05
Cm	0±0	0±0	0±0	0±0	40±0
Tr	0±0	0±0	0±0	0±0	43±0.05
Ck	0±0	0±0	0±0	0±0	45±0
Bacterial strains					**Standard (Ciprofloxacin)**
Sa	0±0	0±0	0±0	6±0.05	34±0
St	0±0	0±0	0±0	7±0.05	35±0.11
Kp	0±0	0±0	0±0	6±0.05	37±0.05
At	0±0	0±0	0±0	0±0	35±0.05
Ps	0±0	0±0	0±0	7±0.05	32±0.05
Pa	0±0	0±0	0±0	8±0.11	34±0.05
Xc	0±0	0±0	0±0	6±0.05	31±0.05
Ec	0±0	0±0	0±0	7±0.05	34±0.05
Sp	0±0	0±0	0±0	0±0	36±0.05

Ca: Candida albicans, Cm: Chrysosporium merdarium, Tr: Trichophyton rubrum, Ck: Chrysosporium keratinophilum, 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 4: Methanolic crude extract of *Olea dioica* against test microbial strains

Test organisms	Zone of inhibition in mm (Mean±SD)				
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	
Fungal strains					**Standard (fluconazole)**
Ca	0±0	0±0	0±0	0±0	30±0.05
Cm	0±0	0±0	0±0	0±0	40±0
Tr	0±0	0±0	0±0	0±0	43±0.05
Ck	0±0	0±0	0±0	0±0	45±0
Bacterial strains					**Standard (Ciprofloxacin)**
Sa	0±0	10±0	12±0.05	18±0.05	34±0
St	0±0	11±0.05	10±0.1	18±0.1	35±0.11
Kp	6±0.05	9±0.05	11±0.11	18±0.05	37±0.05
At	0±0	6±0.05	9±0.05	12±0.05	35±0.05
Ps	0±0	6±0.05	9±0.1	13±0.05	32±0.05
Pa	0±0	7±0.05	12±0.05	15±0.05	34±0.05
Xc	0±0	8±1.3	12±0.1	17±0.05	31±0.05
Ec	0±0	8±0.05	13±0.05	19±0.05	34±0.05
Sp	6±0.05	9±0.05	8±0.1	15±0.05	36±0.05

Ca: Candida albicans, Cm: Chrysosporium merdarium, Tr: Trichophyton rubrum, Ck: Chrysosporium keratinophilum, 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.

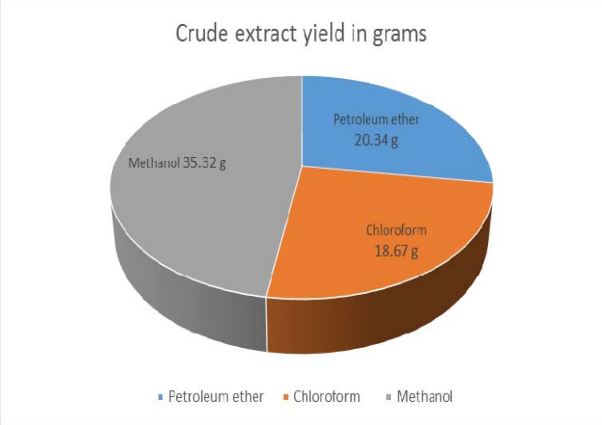

Chart 1: Crude extract yield in different solvent in grams

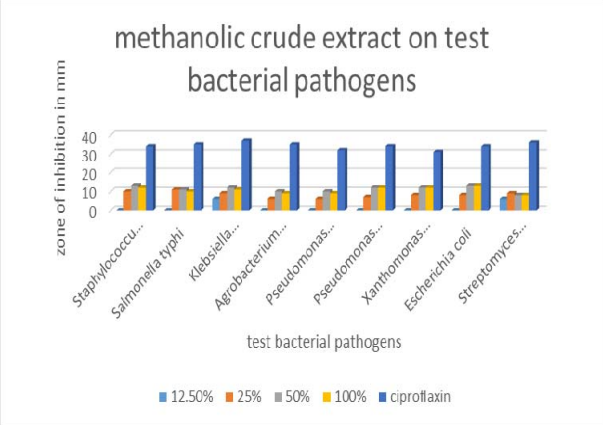

Chart 2: Activity of methanolic crude extract against bacterial pathogens.

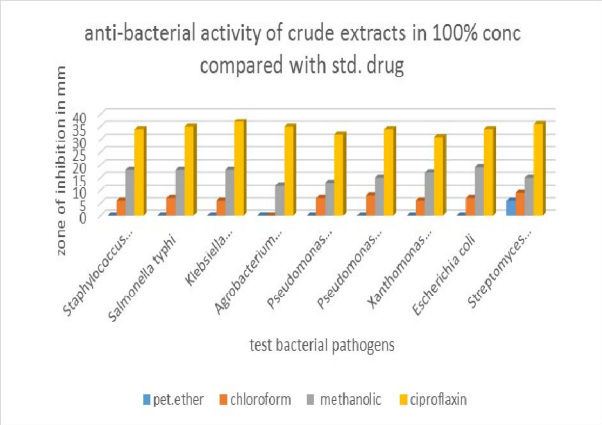

Chart 3: Different crude extracts performance in 100% concentration

Discussion

The experiment revealed that only the methanolic crude extract of *Olea dioica* was effective against bacterial strains but nil antifungal activity. (Table 4 and Chart 3). The methanolic bark crude extract of *Olea dioica* displayed concentration dependent antibacterial activities and this was comparable to that of the reference drug (Table 2, 3 and 4).

Other two extract were showed nil effect on the tested pathogens in all concentrations. (Table 2 and 3).

The methanolic extract shows highest zone of inhibition against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumonia* (table 4).This result shows that the plant methanolic crude extract might be useful in the treatment of these infections. This antibacterial properties may due the presence of flavonoids, glycosides, phenols, alkaloids, saponins and sterols in methanolic crude extract. (Table 1)

Acknowledgement

The authors thankful to department of PG studies and research in Applied Botany, Jnanasahyadri, Shankaraghatta, Kuvempu University for providing facilities to conduct our experimental work. Authors also thankful to Chandrashekar MB, Arun KB, Ashwini HS, Srinivas SG, Nandan patel KJ and Jayashree K Kodiyalmat for the support in conducting experiment.

Reference

1. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts, J Appl. Microbiol. 1999; 86(6):985.
2. Subramani SP, Goraya GS. Some Folklore medicinal plants of Kolli hills Record of a Watti vaidyas Sammelan, J Econ Taxon Bot. 2003; 27(3):665-678.
3. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999; 12(4):564-582.
4. Demain AL, Sanchez S. Microbial drug discovery 80 years of progress, J Antibiot. 2009; 62:5-16.
5. Davies J, Davies D. Origins and evolutions of antibiotic resistance. Microbiol Mol Biol Rev 2010; 74(3):417-433.
6. Ruddock PS, Charland M, Ramirez S, Lopez A, Neil TGH, Arnason JT. Antimicrobial activity of flavonoids from *Piper lanceaefolium* and other Colombian medicinal plants against antibiotic susceptible and resistant strains of *Neisseria gonorrhoeae*. Sexually Transmitted Diseases 2011; 38(2):82-88.
7. Singh B, Singh S. Antimicrobial activity of terpenoids from *Trichodesma amplexicaule* Roth. Phyto-therapy Res 2003; 17(7):814-816.
8. Taleb-Contini SH, Salvador MJ, Watanabe E, ItoIY, de Oliveira DCR. Antimicrobial activity of flavonoids and steroids isolated from two Chromolaena species. Rev Bras Cienc Far, 2003; 39(4):403-408.
9. Mandal P, Sinha BSP, Mandal NC. Antimicrobial activity of saponins from Acacia auriculiformis. Fitoterapia 2005; 76(5):462-465.
10. Nazemiyeh H, Rahman MM, Gibbons S, Nahar L, Delazar A, Ghahramani MA. Assessment of the antibacterial activity of phenyl ethanoid glycosides from *Phlomis lanceolata* against multiple-drug-resistant strains of Staphylococcus aureus, J Nat Med 2008; 62(1):91-95.
11. Pullaiah T. Biodiversity in India. Regency Publications, New Delhi 2006; 4:281-282.
12. Yesodharan K, Sujana KA. Wild edible plants traditionally used by the tribes in Parambikulum wildlife sanctuary, Kerala, India. Natural Product Radiance 2007; 6(1):74-80.
13. Ashwathanarayana R, Raja naika. Comparative study of different solvent extract of *Olea dioica* Roxb. Western Ghats, Karnataka, against selected plant and animal pathogenic bacteria, J Pharm Bio Res. 2015; 3(1):217-222.
14. Ashwathanarayana R, Raja naika. Preliminary Phytochemical and Antifungal Activity of Olea dioica Roxb. Collected from Western Ghats, Karnataka India., Advances in Plant Sciences and Biotechnology, Goa University, 2015.
15. De-Castro MDL, Ayuso LEG. Soxhlet extraction of solid materials an outdated technique with a promising innovative future. Analytica Chimica Acta 1998; 369:1-10.
16. Ajaiyeoba EO. Phytochemical and antimicrobial studies of *Gynandropsis gynandra* and *Buchholzia coriacea* extracts, Afr J Biomed Res. 2000; 3:161-165.
17. Harborne JB. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. (3rdedition). Chapman and Hall Co. New York 1998; 1-302.
18. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall Ltd 1973. 49-188.
19. Prashith Kekuda TR, Vinayaka KS, Poornima G, Dileep N, Rakesh KN, Syed J *et al.* Elemental composition, Antimicrobial and Cytotoxic efficacy of *Olea dioica* Roxb. (Oleaceae), Int J Drug Dev & Res. 2014; 6(1):269-279.