Chemical and Process Engineering Research
ISSN 2224-7467 (Paper) ISSN 2225-0913 (Online)
Vol.24, 2014

Antimicrobial activity of leaf extracts of Jurinea dolomiaea plant against clinical and phytopathogenic bacteria.

S. D Dwivedi ,. Sheeraz Ah. Wagay

Professor & Head, Department of Chemistry, Govt. Benazeer College Bhopal.

Abstract
The ethnobotanical efficacy of Jurinea dolomiaea plant is examined using agar disc diffusion method against clinical bacteria (*Escherichia coli* and *Staphylococcus aureus*) and phytopathogenic bacteria (*Xanthomonas vesicatoria* and *Ralstonia solanacearum*). Leaves were extracted using different solvents such as methanol, ethanol, ethyl acetate and chloroform. Among treatments, maximum in vitroinhibition was scored in methanol extract of which offered inhibition zone of 10, 9, 12 and 12 mm against *E. coli, S. aureus, X. vesicatoria and R. solanaccearum*, respectively, followed by chloroform extract of the same plant leaf with inhibition zone of 8, 4, 4 and 4 mm, respectively. The minimum inhibitory concentration (MIC) value for the clinical bacteria ranged between 0.35 to 4.0 mg/ml and 0.25 to 4.0 mg/ml for phytopathogenic bacteria when tested with all four solvents extracts of *J.dolomiaea*.

Introduction

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine. Thus it is a logical approach in drug discovery to screen traditional natural products. Approximately 20% of the plants found in the world
have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources. It has been reported that between the years 1983 and 1994 (Cragg et al., 1999), the systematic screening of antibacterial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant bacteria. Several workers throughout the world have carried out antimicrobial studies on some medicinal plants including *Betula pendula* (Mukhtar et al., 2002) and *Ageratum houstonianum* (Bowers et al., 1976). According to World Health Organization (Santos et al., 1995) medicinal plants would be the best source to obtain a variety of drugs.

Current advancements in drug discovery technology and search for novel chemical diversity have intensified the efforts for exploring leads from Ayurveda the traditional system of medicine in India. Ayurvedic system of medicine has its long history of therapeutic potential.

The use of plant extracts and phytochemicals both with known antimicrobial properties is of great significance, in the past few years a number of investigations have been conducted worldwide to prove antimicrobial activities from medicinal plants (Alonso-Paz et al., 1995; Nascimento et al., 1990). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization (Santos et al., 1995) medicinal plants would be the best source to obtain a variety of drugs. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are a part of the essential oils (Jansen et al., 1987) as well as tannin (Saxena et al., 1994).

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas et al., 2003). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004). Green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides (Mahajan and Das, 2003). Biopesticides has been suggested as an effecttive substitute for chemicals (Kapoor, 2001). Reports are available on the use of several plant by-products, which posses antimicrobial properties, on several pathogenic bacteria and fungi (Bylka et al., 2004; Shimpi and Bendre, 2005; Kilani, 2006). Here, we evaluate the potential of several plant extracts for antibacterial activity against important human pathogenic and phytopathogenic bacteria.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Jurinea dolomiaea* was collected from Hemalian region of upper Danchigam in south Kashmir about 38- 45 meters above the sea level. The plants were identified taxonomically and authenticated at the

Herbarium, Department of Botany, Kashmir university. Fresh leaves was washed thoroughly 2 - 3 times with running tap water and then with sterile water followed by shade-dried, powdered and used for extraction.

Test microorganisms

Human pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* were collected from GMCmedical college Bhopal India. Plant pathogenic bacteria such as *Xanthomonas vesicatoria* and *Ralstonia solanacearum* were collected from the culture collection of Department of Biotechnology, Barkathullah University Bhopal, India. All the test bacterial species were maintained on nutrient agar media.

Preparation of aqueous plant extracts

25 g of shade dried, powder of plant materials were macerated separately with 50 ml of sterile distilled water using pestle and mortar. The macerate was first filtered through four layer of muslin cloth and then filtrate was centrifuged at 8,000 rpm for 15 min at room temperature. Supernatant was filtered through Whatman No. 1 filter paper and heat sterilized at 120ºC for 30 min. The extract was preserved aseptically in a brown bottle at 4oC until further use.

Preparation of solvent extractions

25 g of shade dried, powder of plant materials were filled separately in the thimble and extracted successively with 150 ml each of methanol, ethanol, ethyl acetate and chloroform using a Soxhlet extractor for 48 h. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extract was weighed and preserved at 4oC in airtight bottles until further use. 1 g of each solvent residue was dissolved in 10 ml of respective solvents were used as the test extracts for antimicrobial activity assay.

Anti-bacterial activity assay

Antibacterial activity of aqueous extract and solvent extracts; methanol, ethanol, ethyl acetate and chloroform was determined by disc diffusion method on nutrient agar medium (Anonymous, 1996). Sterile Whatmann filter discs (6 mm diameter) were made in nutrient agar plate using sterile cork borer (5 mm) and inoculums containing 106 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 µl each of all aqueous and solvent extracts were placed in the discs made in inoculated plates. The treatments also included 50 µl of solvents served as control and chloramphenicol as a standard control. The plates were incubated for 24 h at 37ºC and zone of inhibition if any around the wells were measured in mm (millimeter). Each treatment consists of three replicates and repeated at least twice. Minimum inhibitory concentration (MIC), was determined as the lowest concentration of *J. dolomiaea* plant extracts inhibiting the growth of the organism.

RESULTS

The ethnobotanical efficacy of various solvent extracts of *Jurinea dolomiaea* against both human and plant pathogenic bacteria showed varied level of inhibition (Table 1). Among treatments, maximum in vitro inhibition of tested bacteria *E. coli, S. aureus, X. vesicatoria* and *R. solanaccearum* was scored in methanol extracts which offered inhibition zone of 10, 9, 12 and 12 mm respectively. Further, chloroform extract of *J. dolomiaea* was effective against all four tested bacteria which recorded significant inhibition zone of 12, 11, 7 and 9 mm respectively. Plant extract of *C. odorata* in methanol showed MIC of 2.0 mg/ml against phytopathogenic bacteria *X. vesicatoria* and *R. solanaccearum,* whereas 4.0 mg/ml for clinical bacteria *E. coli* and *S. aureus*. Chloroform leaf extract showed MIC of 4.0 mg/ml against all tested bacteria. Aqueous extract showed MIC of 4.0 mg/ml against *E. coli* and *X. vesicatoria*, whereas MIC of 1.0 and 2.0 mg/ml were found against *S. aureus* and *R. solanaccearum*, respectively (Table 2). The MIC of 2.0 and 4.0 mg/ml was found against all the tested bacteria when ethanol extracts of *C. odorata* were used. The MIC of 4.0 mg/ml was found against *E. coli, X. vesicatoria* and *R. solanaccearum* when ethyl acetate extract was used, whereas MIC of 1.0 mg/ml was found against *S. aureus.*

Table 1. Zone of inhibitory activity (in millimeter) of different plant extracts against clinical and phytopathogenic bacteria.

Source	Extract	E.coli	S. aureus	X. vesicatoria	R. solanacearum
J. dolomiaea	Aqueous	–	–	–	–
	Methanol	10mm	9mm	12mm	12mm
	Ethanol	–	–	–	–
	Ethylacetate	–	–	–	–
	Chloroform	12mm	11mm	7mm	9mm

.

Table 2. Minimum inhibitory concentration (MIC) of *J. dolomiaea* for antibacterial activity.

Source	Extract	MIC(mg/ml)			
		E.coli	S.aureus	X.vesicatoria	R.solanacearum
J.dolomiaea	Aqueous	4.00	1.00	4.00	2.00
	Methanol	4.00	4.00	2.00	2.00
	Ethanol	2.00	4.00	2.00	4.00
	Ethylacetate	4.00	1.00	4.00	4.00
	Chloroform	4.00	4.00	4.00	4.00
	Chloramphenicol	8.00	10.00	9.00	8.00

Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. The methanol, ethanol, ethylacetate and chloroform and aqueous extracts of the leaves of *J.dolomiaea* was subjected to a preliminary screening for antimicrobial activity against two human pathogenic bacteria *E. coli* and *S. aureus* and two phytopathogenic bacteria *X. vesicatoria* and *R. solanacearum*. It was clear from the present results, that both methanol and chloroform leaves extracts of *J.dolomiaea* exhibited pronounced activity against all the tested four bacteria.This tends to show that the active ingredients of the plant parts are better extracted with methanol than other solvents. The methanol extracts contain alkaloids, coumarins and tannins (Okemo, 1996). Coumarins and tannins have antibacterial and antihelminthic properties (Hedberg et al., 1983), also Eloff (1998) and Cowan (1999) found that methanol was more efficient than acetone in extracting phytochemicals from plant materials. The absence of antibacterial activity of chloroform, ethyl acetate and ethanolic extracts of *J.dolomiaea* indicates the insolubility of the active ingredients in these solvents. In general the activities against test bacterial culture used have shown good activity when compared with standard antibiotics. In another research, dichloromethane and aqueous extracts from the leaves as well as ethyl acetate extracts from the flowers have shown antibacterial activity against *Staphylococcus aureus* (Kameda et al., 1987). The minimum inhibitory concentration (MIC) for clinical bacteria was ranged between 0.35 to 4.0 mg/ml and 0.25 to 4.0 mg/ml for phytopathogenic bacteria when tested with all four solvents extracts of *J.dolomiaea*. Various investigators demonstrated that the extract of the leaves of *J.dolomiaea* at low concentrations (from 0.1 to 5 mg/ml) inhibits the growth of *Pseudomonas aeruginosa, E. coli, S. aureus* and *Neisseria gonorrhoea* (Irobi, 1992; Bamba et al., 1993; Caceres et al., 1995). *Jurinea* species (Asteraceae) have been chemically investigated; flavornoids and terpenoids are extensively distributed in this genus (Amaro-Luis and Delgado, 1993; Biller et al., 1994). The presence of these flavonoids in *C. moritziana* could contribute to the observed antibacterial activity (Baez et al., 1998). In the present investigations the antibacterial activity of *J.dolomiaea* against phytopatho-genic bacteria such as *Ralstonia solancearum* and *Xanthomonas vesicatoria* has been demonstrated for the first time .High activity against the Gram-positive organism *E. coli* was found in aqueous and all tested solvent extracts. Similar observations were

reported from nimbolide isolated from neem seed oil showing antibacterial activity against *S. aureus* and Staphylococcus coagulase (Nazma and Rao, 1977). Also antimicrobial effects of neem e *ccus mutans* and *S. faecalis* (Almas, 1999).These might be due to presence of triterpenoids, phenolic compounds, carotenoids, steroids, valavinoids, ketones and tetratriterpenoids azadirachtin (Kraus, 1995). Shariff et al., 2006 reported that *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracted in chloroform were found to inhibit *E. coli* and *X. vesicatoria* at minimum inhibitory concentration (MIC) ranged between 0.25 to 6 mg/ml.

J.dolomiaea methanolic extract posses a broad spectrum of activity against a panel bacteria responsible for the most common bacterial diseases. These primary extracts open the possibility of finding new clinically effective antibacterial compounds. *J.dolomiaea* providing active extract are found in different locations of Hemalia and are well known plants as most of them are used for various medical purposes (Phan et al., 2001). Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within plants and also to determine their full spectrum of efficacy. However the present study of in vitro antibacterial evaluation of plants forms a primary platform for further phytochemical and pharmacological studies to discover new antibiotic drugs.

REFERENCES

1. Almas K (1999). The antimicrobial effects of extracts of *Azadirachta indica* (Neem) and *Salvadora persica* (Arak) chewing sticks. Indian. J. Dent. Res. 10: 23-26.
2. Alonso-Paz E, Cerdeiras MP, Fernandez J, Ferreira F, Moyna P, Soubes M, Vazquez A, Veros S, Zunno L (1995). Screening of Uruguayan medicinal plants for antimicrobial activity. J. Ethanopharm. 45: 67-70.
3. Amaro-Luis JM, Delgado P (1993). Flavonoids from the leaves of *Chromolaena subscandens*. J. Nat. Prod. 56: 610-612.
4. Anonymous (1996). The Indian Pharmacopoeia. 3rd edition. Government of India, New Delhi. Ministry of Health and family welfare.
5. Baez DH, de los Ryos C, Crescente O, Caserta A (1998). Antibacterial and chemical evaluation of *Chromolaena moritziana*. J. Ethnopharm. 59: 203-206.
Sukanya et al. 6681
6. Bamba D, Bessiere JM, Marion L, Pelissier Y, Fouraste I (1993). Essential oil of *Eupatorium odoratum*. Plant Med. 59: 184-185.
7. Benkeblia N (2004). Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and Garlic (*Allium sativum*). Lebensmwissu-Technol. 37: 263-268.
8. Biller A, Boppre M, Witte L, Hartmann T (1994). Pyrrolizidine alkaloids in Chromolaena odorata. Chemical and chemoecological aspects. Phytochem. 35: 615-619.
9. Bowers WS (1976). Discovery of insect juvenile hormone in plants (agretochrome I, II). Sci. 195: 542-547.
10. Bylka W, Szaufer-Hajdrych M, Matalawska I, Goslinka O (2004). Antimicrobial activity of isocytisoside and extracts of Aquilegia *vulgaris* L. Lett. Appl. Microbiol. 39: 93-97.
11. Caceres A, Menendez H, Mendez E, Cohobon BE, Samayoa E, Jauregui E, Peralta Carrillo G (1995). Antigonorrheal activity of plants used in Guatemala for the treatment of sexually transmitted diseases. J. Ethnopharm. 48(2): 85-88.
12. Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12: 564-582.
13. Cragg GM, Boyd MR, Khanna R, Kneller R, Mays TD, Mazan KD, Newman DJ, Sausville EA (1999). International collaboration in drug discovery and development: the NCl experience. Pure Appl. Chem. 71: 1619-1633.
15. Eloff JN (1998). Which extractan should be used for screening and isolation of antimicrobial components from plants. J. Ethnopharm. 60:1-8.
16. Hedberg I, Hedberg O, Madati P, Mshigeni KE, Mshiu EN, Samuelsson G (1983). Inventory of plants used in traditional medicine in Tanzania. II. Plants of the family Dilleniaceae to Opiliaceae. J. Ethnopharm. 9:105-128.
17. Hopper M, Kirby GC, Kulkarni MM, Kulkarni SN, Nagasampagi BA, O'Neill MJ, Philipson JD, Rojatkar SR, Warhurst DC, (1990). Antimalarial activity of parthenin and its derivatives. Eur. J. Med. Chem. 25: 717-723.
18. Irobi ON (1992). Activities of *Chromolaena odorata* (Compositae) leaf extract against Pseudomonas aeruginosa and Streptococcus faecalis. J. Ethanopharm. 37: 81-83.
19. Jansen AM, Cheffer JJC, Svendsen AB (1987). Antimicrobial activity of essential oils: A 1976-1986 literature review. Aspects of test methods. Plant. Med. 40: 395-398.
20. Kameda K, Takaku T, Okuda H, Kamura Y, Hatano T, Agata T, Arichi S,
(1987). Inhibitory effects of various flavonoids isolated from leaves ofpersimmon on angiotensin-converting enzyme activity. J. Nat. Prod. 50: 680-683.
21. Kanchan SD (1975). Growth inhibitors from *P. hysterophorus*. Curr. Sci.44: 358-361.
22. Kapoor A (2001). Neem: The wonder plant. Pesticides Inform. 27: 33-34.

23. Kilani AM (2006). Antibacterial assessment of whole stem bark of Vitex doniana against some Enterobactriaceae. Afr. J. Biotechnol. 5: 958-959.
24. Kraus W (1995). In the Neem Tree: Source of Unique Natural products for Integrated Pest Management, Medicine, Industry and other purposes (ed. Schmutterer H). pp. 35-88.
25. Mahajan A, Das S (2003). Plants and microbes- Potential source of pesticide for future use. Pesticides Inform. 28(4): 33-38.
26. Mukhtar MH, Ansari SH, Ali M, Wani FA (2002). Antimicrobial activity of
Betula pendula. Hamdard Medicus. 45: 41-43.
27. Nascimento SC, Chiappeta A, Lima RM (1990). Antimicrobial and cytotoxic activities in plants from pernambuco, Braz. Fitoter. 61: 353-355.
28. Nazma BSV, Rao JM (1977). Antifungal Activity of Gedunin. Curr. Sci.46: 714-715.
29. Okemo PO (1996). Antimicrobial efficacy of selected medicinal plants used by Kenyan Herbal doctors. Ph.D. thesis, Kenyatta University of Nairobi, pp. 173-90.
30. Phan TT, Hughes MA, Cherry GW (2001). Effects of an aqueous extract from the leaves of *Chromolaena odorata* (Eupolin) on the proliferation of human keratinocytes and on their migration in an *in vitro* model of reepithelialization. Wound. Rep. Reg. 9: 305-313.
31. Rojas R, Bustamante B, Bauer J (2003). Antimicrobial activity of selected Peruvian medicinal plants. J. Ethanopharm. 88: 199-204.6682 Afr. J. Biotechnol.
32. Santos PRV, Oliveira ACX, Tomassini TCB (1995). Control microbiogicode
productos. Fitoterapicos. Rev. Farm. Bioquim. 31: 35-38.
33. Saxena G, McCutcheon AR, Farmer S, Towers GHN, Hancock REW (1994). Antimicrobial constituents of *Rhus glabra*. J. Ethanol. Pharm. 42: 95-99.
34. Shariff N, Sudarshana MS, Umesha S, Hariprasad P (2006). Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. Afr. J. Biotech. 5(10): 946-950.
35. Sharma GL, Bhutani KK (1998). Plant Based Antiamoebic Drugs; Part II. Amoebicidal Activity of Parthenin Isolated from *Parthenium hysterophorus*. Plant Med. 54: 120-121.
36. Shimpi SR, Bendre RS (2005). Stability and antibacterial activity of aqueous extracts of *Ocimum canum* leaves. Indian Perfumer. 49(2): 225-229.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar