Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 12, No. 6, p. 139-146, 2018
http://www.innspub.net

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RESEARCH PAPER
OPEN ACCESS

In vitro antibacterial potential of *Withania coagulans* Dunal (Solanaceae)

Abd Ullah*, Adnan Khan

School of life sciences, Northeast Normal University, Changchun, Jilin 130024, P. R. China

Article published on June 21, 2018

Key words: Inhibition potential, Well diffusion assay, *W. coagulans*, Bacteria.

Abstract

The present study was conducted to evaluate the antibacterial potential of *W. coagulans*Dunal. Using agar well diffusion method. Three different crude extracts (ethyl acetate, chloroform and aqueous) of leaves stem and roots were tested against four pathogenic bacterial strains at four different concentrations (0.5, 1.0, 1.5, 2.0 mg/ml). Results show that maximum inhibition had been shown by *W. coagulans*ethyl acetate root extract at 2mg/ml concentration with 24mm, 20mm, 19mm and 23mm zone of inhibition against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Klebsiella pneumonia* respectively. Chloroform leaves extracts were also most effective against all the four pathogenic bacterial strains. Minimum inhibition potential had been shown by aqueous leaves extract (2mg/ml) with 3-11mm zone of inhibition against all tested pathogenic bacterial strains. Furthermore, ethyl acetate and aqueous stem extract (2mg/ml) had almost similar effect against *S. aureus* (18.35 and 18.02mm inhibition zone). Chloroform leaves extract (2mg/ml) and ethyl acetate stem extract had considerable inhibition against the selected bacterial pathogens. Chloramphenicol (standard antibiotic) was much effective against *S. aureus* and *K. pneumonia*with 26.69mm and 25.46 mm zone of inhibition. The overall study determined the medicinal importance of *W. coagulans*.

***Corresponding Author:** Abd Ullah ✉ Abdullahbotany0987@gmail.com

Introduction

Bacteria is the most common pathogen responsible for various infectious diseases worldwide killing more than 50,000 people every day. Common pathogenic bacteria are *Escherichia coli*, *S. aureus,* and *Salmonella* species. These bacteria cause several infectious diseases in children's and adults (Bonjor, 2004). Antibiotics have saved millions of human lives against infectious diseases throughout the world. But recently it has been noted that efficacy of many existing antibiotics is being threatened by alarming increasing the number of drug-resistant bacteria (Ahmad and Beg, 2001). To overcome this panic researchers are trying to develop novel means of fighting bacterial pathogens. One attractive approach is to use the plant or plant parts as a potential antimicrobial agent to fight such bacterial strains and their infectious diseases. A large number of therapeutic drugs, obtained from the plant are used for different infectious disorders (Kunin, 1993). Natural products obtained from plants have pharmaceutical and therapeutic potentials due to the presence of phytochemicals (Scheck *et al*., 2006). These important therapeutic features are anti-inflammatory, antibacterial, antifungal, anticancer, antioxidants, antidiabetic, analgesic (Erasto *et al*., 2005; Shad *et al*., 2013), hypolipidemic, central nervous system depressant, hepatoprotective, antitumor, immuno-suppressive, wound healing and cytotoxic (Gupta and Keshari, 2013). These phytochemicals are commonly known as plant metabolites. These plant metabolites are divided into primary and secondary metabolites (Agosta, 1996; Ramasamy *et al*., 2012). Secondary metabolites are those compounds produced by plants that are not directly essential for basic photosynthetic or respiratory metabolism. Secondary metabolites are alkaloids, essential oils, terpenoids, flavonoids, glycosides and phenolic compounds. These are excellent therapeutic agents and can be used as medicines against a number of infectious diseases due to their antimicrobial actions (Harborne, 1973; Heinrich *et al*., 1998; Edeoga *et al*., 2005; Desideri *et al*., 2010; Gupta *et al*., 2011). Secondary metabolites are also important for plants as many of them have a role in defense against pathogens, pests, and herbivores. Extensive work has been conducted for evaluating the therapeutic values of medicinally important plants (Qin and Xu, 1998; Dias *et al*., 2012). Today a large number of plant-based traditional medicine are developed from plants which are a real substitute for the treatment of human and animal ailments (Wright, 2005). *W. coagulans* belonging to the family Solanaceae (Shahid *et al*., 2013). It is a rigid small shrub and very well known for its ethnopharmacological properties(Kirthikar and Basu, 1933). It is common in Pakistan, Iran, Afghanistan, and India. Fruits of *W. coagulans*have milk-coagulating properties (Naz,2002). This plant having ananti-inflammatory effect (Budhiraja *et al*., 1977) and also possesses anti-hyperglycemic activity in rats and anti-dyslipidemia effect on mice (Maurya *et al*., 2008).

The twigs of this plant are chewed for teeth cleaning and the smoke is inhaled for toothache relief (Kirthikar and Basu, 1933). The present study was aimed to evaluate the inhibition potential of *W. coagulans* Dunal. against four pathogenic bacterial strains.

Materials and methods

Plant samples collection

Plants samples (leaves, stem, and roots) of *W. coagulans* Dunal were collected from Tehsil Tangi, District Charsadda, Pakistan (34-03' and 34-38' north latitudes and 71-28' and 71-53' east longitude).

Experimental site

The present work was conducted at the laboratory of plant sciences, Department of Botany, Bacha University Charsadda, Khyber Pakhtunkhwa Pakistan.

Plant sample processing

The plants were washed properly with sterilized water and shad dried for 3 weeks at 25°C. The various parts of the selected medicinal plants were grounded into

powder form using homogenizer. Powders were stored at room temperature for experimental purposes.

Extraction

The plant parts of 50g were soaked in 200 ml chloroform, ethyl acetate and water using clean and sterilized beakers then after incubated at room temperature (25°C) for 02 weeks. The mixture was filtered twice after 14 days of extraction using Whatman-41 filter paper and the extract was reduced till dryness via rotary evaporator.

Bacterial strains used

Four pathogenic bacterial strains were used in the study. Among the selected pathogenic strains *S. aureus* and *B. subtilis* were gram-positive whereas; *K. pneumoniae* and *P. aeruginosa*were gram negatives. The growth of bacterial pathogen was maintained on nutrient agar at 4°C.

Positive control

Chloramphenicol (1.0 mg/ml) was used as a positive control against bacterial strains.

Assay for antibacterial activity

Antibacterial potential of the selected plant samples was evaluated using the method of Carron *et al,* 1987; for which agar medium (nutrient) was prepared by dissolving 2.30g of nutrient agar in 100ml of distilled water then after; pH was maintained 7.0 and sterilized in an autoclave at 121°C. Media was allowed to cool till 45°C then added 75ml of it in Petri dishes and solidify. Sterile cork borer of 5mm was used for the preparation of four wells per plate. 100µl of plant extract was added in particular wells through a micropipette. These Petri dishes were stored at 37°C in an incubator for 24 hours. After incubation zone of inhibitions was measured in millimeter (mm).

Statistical analysis

The clear zones of inhibition were measured in millimeter. All the data values are the means of three replicates which were tubulated as a Mean ± standard deviation.

Results

Antibacterial potential against S. aureus

Table 1 reveals that maximum inhibition against *S. aureus* has been shown by ethyl acetate and chloroform root extract (2mg/ml concentration) with an inhibition zone of 24.02 and 22.69mm respectively. Ethyl acetate stem extract (2mg/ml concentration) had considerable inhibition zone recorded as 20.68mm. Furthermore, ethyl acetate and aqueous stem extract had almost similar inhibition potentials. *S. aureus* was least sensitive to aqueous leaves extract (8.02mm inhibition zone) as compared to stem and root aqueous extract.

Table 1. Antibacterial activity of *W. coagulans* Dunal. against *S. aureus.*

Solvent extracts	2 mg/ml	1.5 mg/ml	1 mg/ml	0.5 mg/ml
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Chloramphenicol 1.0mg/ml	26.69 ±0.13	25.74 ±0.06	22.99 ±0.06	21.13 ± 0.33
Chloroform leaves extract	19.69 ±0.09	17.74 ±0.02	11.99 ±0.04	10.80 ±0.58
Ethyl acetate leaves extract	14.36 ±0.20	10.08 ±0.58	7.32 ±0.59	7.13 ±0.33
Aqueous leaves extract	8.02 ±0.16	7.74 ±0.35	5.32 ±0.36	5.13 ±0.03
Chloroform stem extract	18.36 ±0.08	13.74 ±0.03	9.32 ±0.07	7.80 ±0.21
Ethyl acetate stem extract	20.69 ±0.05	16.08 ±0.04	11.32 ±0.01	9.47 ±0.88
Aqueous stem extract	18.02 ±0.08	16.74 ±0.03	11.32 ±0.04	9.47 ±0.33
Chloroform root extract	22.69 ±0.04	21.41 ±0.07	16.99 ±0.01	14.80 ±0.16
Ethyl acetate root extract	24.02 ±0.08	22.74 ±0.07	17.99 ±0.06	13.80 ±0.67
Aqueous root extract	17.02 ±0.33	14.08 ±0.02	8.32 ±0.03	6.47 ±0.07

Chloramphenicol= Standard drug, Mean ± SD= Mean zone of inhibition in mm and standard deviation.

The overall inhibition was concentration-dependent, showing a progressive decrease with a decrease in concentration from 2mg/ml to 0.5mg/ml concentration.

Antibacterial potential against P. auregonosa

The *W. coagulans* crude extract were least effective against *P. auregonosa* as compared to other bacterial strains. As shown in Table 2, aqueous leaves extract showed minimum inhibition of 3.99 mm at 2mg/ml concentration. Ethyl acetate and chloroform root extract showed tremendous inhibition against *P. auregonosa* recorded as 19.99 and 18.66 mm respectively. Chloroform leaves extract and ethyl acetate stem extract also had a considerable effect. The overall inhibition was concentration dependent.

Table 2. Antibacterial activities of *W. coagulans* Dunal. against *P. auregonosa*.

Solvent extracts	2 mg/ml	1.5 mg/ml	1 mg/ml	0.5 mg/ml
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Chloramphenicol 1.0mg/ml	22.66 ±0.07	23.06 ± 0.07	19.43 ± 0.04	18.68 ± 0.08
Chloroform leaves extract	15.66 ± 0.07	15.06 ± 0.10	8.43 ± 0.05	8.35 ± 0.04
Ethyl acetate leaves extract	10.33 ± 0.88	7.39 ± 0.33	3.76 ± 0.18	4.68 ± 0.38
Aqueous leaves extract	4.00 ± 0.41	5.06 ± 0.35	1.76 ± 0.26	2.68 ± 0.10
Chloroform stem extract	14.33 ± 0.06	11.06 ± 0.09	5.76 ± 0.10	5.35 ± 0.31
Ethyl acetate stem extract	16.66 ± 0.04	13.39 ± 0.12	7.76 ± 0.15	7.02 ± 0.19
Aqueous stem extract	14.00 ± 0.06	14.06 ± 0.10	7.76 ± 0.07	7.02 ± 0.07
Chloroform root extract	18.66 ± 0.13	18.73 ± 0.10	13.43 ± 0.14	12.35 ± 0.09
Ethyl acetate root extract	20.00 ± 0.03	20.06 ± 0.10	14.43 ± 0.10	11.35 ± 0.07
Aqueous root extract	13.00 ± 0.11	11.39 ± 0.06	4.76 ± 0.10	4.02 ± 0.06

Chloramphenicol= Standard drug, Mean ± SD= Mean zone of inhibition in mm and standard deviation.

Antibacterial potential against B. subtilis

As shown in Table 3, maximum inhibition zones have been shown by *W. coagulans* ethyl acetate and chloroform root extract (2mg/ml concentration) recorded as 18.79 and 17.45mm respectively. *B. subtilis* was least affected by *W. coagulans* aqueous leaves extract (2.79mm).

The considerableeffect was shown by ethyl acetate stem extract and chloroform leaves extract. The overall inhibition potential decrease with a decrease in concentration from 2mg/ml to 0.5mg/ml.

Antibacterial potential against K. pneumonia

Table 4 reveals that *K. pneumonia*was most susceptible to ethyl acetate and chloroform root extract 2mg/ml concentration with inhibition zone recorded as 22.80 and 21.46mm respectively. The minimum inhibition has been recorded as 6.80mm for aqueous leaves extract 2mg/ml concentration. Considerable inhibition shown by ethyl acetate stem extract and chloroform leaves extract recorded as 19.46 and 18.46mm respectively.

The overall inhibition of *K. pneumonia* was concentration dependent. The inhibition values progressively decrease with a decrease in inhibition, indicating that the inhibition potential was concentration dependent. Comparison of the inhibition potential of the crude extracts (Ethyl acetate and chloroform root extracts) with the standard drug has been shown in Fig. 1.

Standard drug (Chloramphenicol) has tremendous effect against all the pathogenic bacterial strains, followed by ethyl acetate and chloroform root extracts respectively.

Discussion

For the past two decades, there has been an increasing interest in the investigation of the various extract obtained from traditional medicinal plants as potential sources of new antimicrobial agents (Bonjar *et al*., 2004). Therefore, the present work was conducted to screen *in vitro* the antibacterial potential of *W. coagulans* against four pathogenic bacterial strains.

Table 3. Antibacterial activity of *W. coagulans* Dunal. against *B. subtilis*.

Solvent extracts	2 mg/ml	1 mg/ml	1.5 mg/ml	0.5 mg/ml
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Chloramphenicol 1.0mg/ml	21.46 ±0.62	20.44 ±0.40	21.81 ±0.47	17.79 ±0.35
Chloroform leaves extract	14.46 ±0.49	9.44 ±0.49	13.81 ±0.35	7.46 ±0.23
Ethyl acetate leaves extract	9.13 ±0.49	4.77 ±0.46	6.14 ±0.92	3.79 ±0.64
Aqueous leaves extract	2.79 ±0.91	2.77 ±0.26	3.81 ±0.58	1.79 ±0.49
Chloroform stem extract	13.13 ±0.99	6.77 ±0.54	9.81 ±0.32	4.46 ±0.58
Ethyl acetate stem extract	15.46 ±0.78	8.77 ±0.25	12.14 ±0.45	6.13 ±0.43
Aqueous stem extract	12.79 ±0.59	8.77 ±0.60	12.81 ±0.54	6.13 ±0.56
Chloroform root extract	17.46 ±0.59	14.44 ±0.77	17.48 ±0.47	11.46 ±0.38
Ethyl acetate root extract	18.79 ±0.21	15.44 ±0.35	18.81 ±0.52	10.46 ±0.49
Aqueous root extract	11.79 ±0.88	5.77 ±0.59	10.14 ±0.61	3.13 ±0.55

Chloramphenicol= Standard drug,Mean ± SD= Mean zone of inhibition in mm and standard deviation(SD).

The present work has shown that the plant crude extract (Ethyl acetate, chloroform and aqueous) inhibited bacterial growth but their effectiveness varied with solvent, their concentration, and the bacterial strain used. Ethyl acetate and chloroform extract of *W. coagulans* had the best antibacterial activity against *P. aeruginosa, B. subtilis, S.aureus* and *K. pneumonia*at high concentration (2mg/ml) which are supported by the work of (Mughal *et al*., 2010).

Table 4. Antibacterial potentials of *W. coagulans* Dunal. against *K. pneumonia*.

Solvent extracts	2 mg/ml	1 mg/ml	1.5 mg/ml	0.5 mg/ml
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Chloramphenicol -1.0mg/ml	25.46 ± 0.43	19.98 ± 0.49	23.58 ± 0.81	16.68 ± 0.43
Chloroform leaves extract	18.46 ± 0.72	8.98 ± 0.49	15.58 ± 0.61	6.35 ± 0.61
Ethyl acetate leaves extract	13.13 ± 0.67	4.31 ± 0.44	7.91 ± 0.95	2.68 ± 0.61
Aqueous leaves extract	6.80 ± 0.57	2.31 ± 0.64	5.58 ± 0.70	0.68 ± 0.33
Chloroform stem extract	17.13 ± 0.35	6.31 ± 0.55	11.58 ± 0.56	3.35 ± 0.31
Ethyl acetate stem extract	19.46 ± 0.53	8.31 ± 0.49	13.91 ± 0.59	5.02 ± 0.44
Aqueous stem extract	16.80 ± 0.60	8.31 ± 0.51	14.58 ± 0.90	5.02 ± 0.38
Chloroform root extract	21.46 ± 0.68	13.98 ± 0.46	19.24 ± 0.53	10.35 ± 0.40
Ethyl acetate root extract	22.80 ± 0.61	14.98 ± 0.62	20.58 ± 0.56	9.35 ± 0.38
Aqueous root extract	15.80 ± 0.61	5.31 ± 0.42	11.91 ± 0.74	2.02 ± 0.33

Chloramphenicol= Standard drug, Mean ± SD= Mean zone of inhibition in mm and standard deviation(SD).

They reported that the *W. coagulans*seed crude methanol extract showed good antibacterial activity against *S.aureus, B. subtilis* but was moderately active against and *P. aeruginosa*. The volatile oil from the fruits of *W. coagulans had also* shown antibacterial activity against *S. aureus* and *Vibrio cholera* (Khan *et al*., 1993; Choudhary *et al*., 2005) also in concord with the present work. Results also showed that ethyl acetate and chloroform root extract at 2mg/ml inhibited the bacterial strains at its maximum, which is supported by the work of (Gain and Budhiraja, 1967). They demonstrated the

antibacterial property of leaves extract of *W.coagulans*. (Sudhanshu *et al*., 2012) investigated the phytochemical screening and antibacterial activity of different extract of *W. coagulans*fruits against various bacterial pathogen's such as *Shigella flexneri, S. aureus, Salmonella typhi, P. aeruginosa, K. pneumonia, Proteus vulgaris, Enterobacter aerogenes* and showed that chloroform fruit extract showed maximum inhibition against *K. pneumonia* (19mm) which is in support of the present experiment in which the chloroform root and leaves extract showed maximum inhibition against *K. pneumonia* with 21.46 and 18.46mm zone of inhibition.

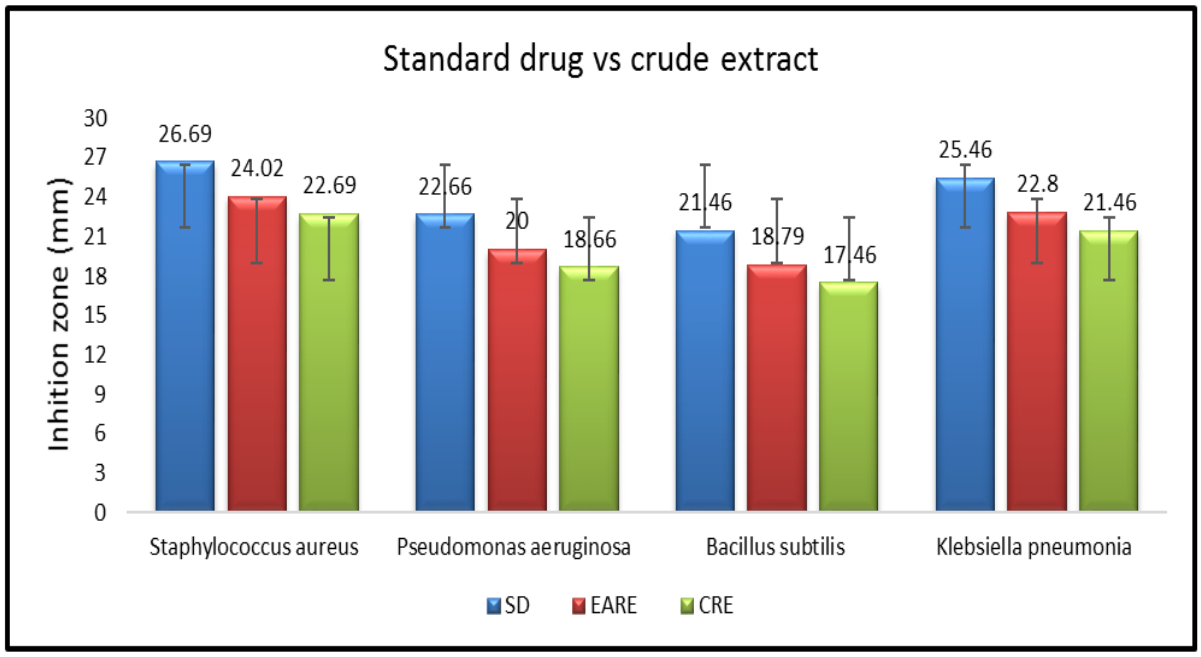

Fig. 1. Comparison of the inhibition potential of ethyl acetate and chloroform root extracts with a standard drug, Key; SD= standard drug (chloramphenicol), EARE = Ethyl acetate root extract, CRE= Chloroform root extract.

Conclusion

Ethyl acetate root extract showed maximum inhibition potential of 24.02mm and 22.80mm against *S. aureus* and *K. pneumonia* respectively, while minimum inhibition has been shown by aqueous leaves extract against *B. subtilis* recorded as 2.79 mm at 2mg/ml concentration.

Acknowledgments

Special thanks to my dear friends Adan Farid, Irshad Ahmad and Mubashir Khan for helping me in plant materials collection. I am also grateful to the Department of Botany, Bacha Khan University Charsadda, KP, Pakistan for providing the laboratory facilities.

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