EVALUATION OF ANTIMICROBIAL PLANT METABOLITES FROM *ACHYRANTHES ASPERA* AGAINST MULTIDRUG RESISTANT CHRONIC WOUND ISOLATES

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ABSTRACT

Wounds are increasing burden to healthcare systems as the availability of drugs capable of stimulating the process of wound repair is still limited to treat the patient. Bacterial infection of wound can impede the healing process and lead to life threatening complications. Therefore, novel antibiotics of plant origin are necessary in order to eliminate the threat of the infections. The aim of this study was to investigate the inhibitory activity of leaf extract of *Achyranthes aspera* against Chronic Wound Isolates (CWI) using different solvents of increasing polarity such as Cyclohexane, Toluene, Benzene, Chloroform, Petroleum ether, Diethyl ether, Aceto nitrile, Propanol, Acetic acid, Methanol and Water. The CWI are found to be Multi Drug Resistant (MDR) against commercial antibiotics Tetracycline, Ampicillin, Vancomycin and Colistin. The profound antimicrobial activity using Disk Diffusion Method was found for Cyclohexane extract having a zone of inhibition of 2.5cm in Diameter. The Minimum Inhibitory Concentration (MIC) for Cyclohexane was 0.5mg/ml. The inhibitory potential of plant metabolites was again evidenced by Growth Curve analysis. The TLC analysis of Cyclohexane extract in Cyclohexane: Acetonitrile: Propanol solvent system revealed a single compound of R_F Value 0.71. The functional group present in the Cyclohexane extract was analysed by FTIR. The above studied results supported the fact that Cyclohexane extract of *Achyranthes aspera* has potential antimicrobial activity against Multi Drug Resistant CWI. The isolation of Active compounds from Cyclohexane extract of *Achyranthes aspera* will exhibit a promising effect in treating chronic wound injuries.

KEY WORDS: Antimicrobial activity, Multi Drug Resistant strain, MIC, Growth Curve, FTIR

1. INTRODUCTION

In this world, the nature has been a source of medicinal agents from thousands of years and an impressive number of modern drugs have been isolated from natural sources. Medicinal plants are known to treat many infections and disease for the man kind (Zaiden, 2005). Plants and its compounds play a major role in primary health care as therapeutic remedies. Wounds and wound injuries are the cause for infections and leads to life threatening diseases. Infectious diseases are the world's leading cause of premature deaths (Emori and Gaynes, 1993). They also cause exsposure of subcutaneous tissue following a loss of skin integrity (i.e, a wound) provides a moist, warm, and nutritious environment that is conducive to microbial colonization and proliferation. The wound infection may be Acute, Chronic and it may be due to surgery, Bites, and Burns. In case of wound healing; one of medicinal plant used as therapeutics is *Achyranthes aspera* (Amaranthaceae) and is a habitat of Asia, South America and Africa. In traditional medicinal system, *A. aspera* is known for diuretic, hepatoprotective and emmenagogue properties and used to cure several diseases viz., malarial fever, dysentery, asthma, hypertension and diabetics. Most recently, *A. aspera* is widely studied for its medicinal properties and reported to have immunostimulatory properties (Rao, 2002), wound healing activity, antioxidant activity, h**a**emolytic activity (Priya, 2010), anti-inflammatory (Kumar, 2009), antibacterial activity (Alam, 2009) and antifungal activity (Elumalai, 2009). The antimicrobial drugs are commonly used in the treatment of infectious diseases (Lakshmi, 2006). The development of drug resistance as well as the appearance of undesirable side effects is common in certain antibiotics (Okemo, 2003) and it has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages.

The aim of this present study is to evaluate the antimicrobial activity of plant metabolites from *Achyranthes aspera* against Chronic wound Isolates.

2. MATERIALS AND METHODS

2.1. Plant Collection and Pretreatment: *Achyranthes aspera* leaves were collected from Aruppukottai, Virudhunagar (Dt), Tamil Nadu. The leaves were shade dried and powdered by the electrical mixer and stored in airtight container at 37^0C.

2.2. Preparation of Plant extracts: 5g of plant leaf powder was soaked and crushed with 50ml of different solvents of increasing polarity (Cyclohexane, Toluene, Benzene, Chloroform, Petroleum ether, Diethyl ether, Aceto nitrile, Propanol, Acetic acid, Methanol and Water) and was boiled for 12hrs at the boiling temperature of solvents. After boiling the extract was filtered with Whatman no 1 filter paper and the filterate was dried in Hot air oven to obtain the powdered plant extracts. 1mg of plant extract was dissolved in 1ml of DMSO and it was used for further analysis.

2.3. Chronic Wound Sample Collection: The Chronic Wound Sample was collected from a person who have suffered from Chronic wound infection in Government Hospital, Madurai. The collected sample was stored in saline water and it was inoculated in Nutrient broth which is kept in shaker at 30^0C.

2.4. Multi Drug Resistance Behavior of Chronic Wound Sample: Chronic Wound isolate was checked for its antibiotic resistance against various commercial antibiotics such as Tetracycline, Ampicillin, Kanamycin, Colistin, Vancomycin through Disc Diffusion Method. The Log phase culture of chronic wound isolate was inoculated in nutrient agar plate and incubated at 37˚C. Zone of inhibition formed for the commercial antibiotics were measured in diameter.

2.5. Anti-microbial activity

2.5.1. Well Diffusion Method: Spread culture of chronic wound isolate was made in a nutrient agar plate. Wells of 0.5cm were made in the agar plate and Plant Extracts of different solvents were loaded in the wells. Solvents were loaded in separate wells as positive control. The plates were incubated at 37 ˚C for 24hrs. Then, the zone of inhibition was measured in diameter.

2.5.2. Determination of MIC: Plant extracts of varied concentration (0.25mg/ml, 0.5mg/ml, 0.75mg/ml and 1mg/ml) exhibiting potent Antimicrobial activity was dissolved in DMSO. The active log phase culture of Chronic Wound isolate was used to screen Minimum Inhibitory Concentration (MIC) of plant extracts by Agar Well Diffusion Method.

2.5.3. Growth Curve: The inhibitory effect of Potent antimicrobial plant extracts of varied concentration against Chronic Wound Isolate was studied through growth curve analysis. The powder form of 1% potent antimicrobial plant extract was dissolved in Nutrient broth (Himedia, India) of 200ml containing 1% inoculums.

2.5.4. Thin layer chromatography (TLC) analysis: TLC analysis was done for the potent plant extract using mixed and gradient solvent system in a trial and error process. The solvent system choosen was Cyclohexane: Acetonitrile: Propanol (1:1:1) and Methanol (25%, 75%, 100%). The TLC was performed and the plates were visualized under White and UV light illimination for the identification of Compounds in the potent plant extract.

2.5.5. Fourier Transforms Infrared Spectroscopy (FTIR) Analysis: The FT- IR spectra of Cyclohexane extracts were measured on Shimadzu FT-IR 8000 series instrument. The sample was grounded with IR grade potassium bromide (KBr) (1:10) pressed into discs under vacuum using spectra lab pelletiser. The IR spectrum was recorded in the region 4000-400 cm^{-1}.

3. RESULTS AND DISCUSSION

3.1. Screening of Potent Antimicrobial Plant Metabolites: The screening of potent antimicrobial plant metabolite from *Achyranthes aspera* is achieved through different solvents of increasing polarity. The polar solvent Cyclohexane showed potent antimicrobial activity against CWI with a zone of inhibition of 2.5 cm in diameter than other solvents such as Toluene, Benzene, Chloroform, Petroleum ether, Diethyl ether, Acetonitrile, Propanol, Acetic acid, Methanol and Water (Listed in Table 1). The CWI were MDR against commercial antibiotics Tetracycline, Ampicillin, Vancomycin and Colistin. The Minimum Inhibitory concentration for Cyclohexane Extract against CWI was found to be 0.5mg/ml with a zone of inhibition of 1.8cm in diameter (Table 2). Also the Growth curve analysis of Cyclohexane extract shows, effective inhibition (6 fold) of microbial growth at the log phase. This evidences its potent antimicrobial activity against Multi Drug Resistant Chronic Wound Isolates. These results prove the fact that Cyclohexane extract was potent antimicrobial inhibitor.

Table 1: Antimicrobial activity of different solvents against Chronic Wound Isolates

S.NO.	SOLVENTS	ZONE OF INHIBITION IN DIA (cm)
1.	Control	-
2.	Cyclohexane	2.5
3.	Toluene	1.7
4.	Benzene	1.5
5.	Chloroform	-
6.	Petroleum ether	-
7.	Diethyl ether	-
8.	Aceto nitrile	-
9.	Propanol	1.3
10.	Acetic acid	-
11.	Methanol	-
12.	Water	-

Table 2: MIC for Cyclohexane extract against Chronic Wound Isolates

S.No	Concentration of Cyclohexane (mg/ml)	Zone of Inhibition in Diameter (cm)
1.	0.25	-
2.	0.5	1.8
3.	0.75	2.3
4.	1.0	2.7

Table 3: FTIR analysis of Cyclohexane extract

S.NO	ABSORPTION FREQUENCY REGION	WAVE NUMBER Cm^{-1}	BOND	MAJOR FUNCTIONAL GROUP
1.	3500-3200 (s,b)	3404.36	O-H stretch, H-bonded	Primary and secondary amines, amides
2.	3000-2850 (m)	2924.09	C-H stretch	Alkanes
3.	3000-2850 (m)	2854.65	C-H stretch	Alkanes
4.	1760-1665 (s)	1739.79	C=O stretch	Carbonyls(general)
5.	1680-1640 (m)	1651.07	-C=C-stretch	Alkenes
6.	1650-1580 (m)	1635.64	N-H bend	Primary amines
7.	1550-1475 (s)	1519.91	N-O asymmetric stretch	Nitro compounds
8.	1470-1450 (m)	1460.11	C-H bend	Alkanes
9.	1500-1400 (m)	1421.54	C-N stretch (in-ring)	Aromatics
10.	1335-1250 (s)	1325.10	C-N stretch	Aromatic amines
11.	1250-1020 (m)	1247.94	C-N stretch	Aliphatic amines
12.	1250-1020 (m)	1247.94	C-N stretch	Aliphatic amines
13.	1320-1000(s)	1109.07	C-O stretch	Alcohols, Carboxylic acids, Esters, Ethers
14.	1320-1000(s)	1033.85	C-O stretch	Alcohols, Carboxylic acids, Esters, Ethers
15.	910-665 (s,b)	898.83	N-H wag	Primary and secondary amines
16.	910-665 (s,b)	829.39	N-H wag	Primary and secondary amines
17.	850-550 (m)	779.24	C-Cl stretch	Alkyl halides
18.	850-550 (m)	671.23	C-Cl stretch	Alkyl halides
19.	700-610 (b,s)	617.22	-C≡C-H:C-H bend	Alkynes
20.	690-515 (m)	516.92	C-Br stretch	Alkyl halides

3.2. TLC analysis: The TLC Analysis of Cyclohexane extract in the solvent system Cyclohexane: Acetonitrile: Propanol (1:1:1) showed a single spot with an R_F value of 0.71 and gives pink color fluorescence under UV light illumination. This result notes that Cyclohexane extract contains a single potent antimicrobial compound.

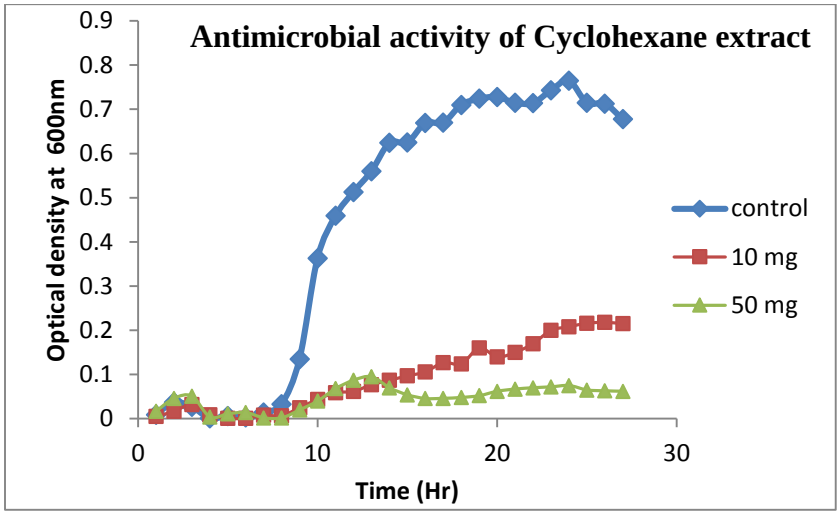

Fig 1, Antimicrobial activity of Cyclohexane extract by Growth Curve Analysis

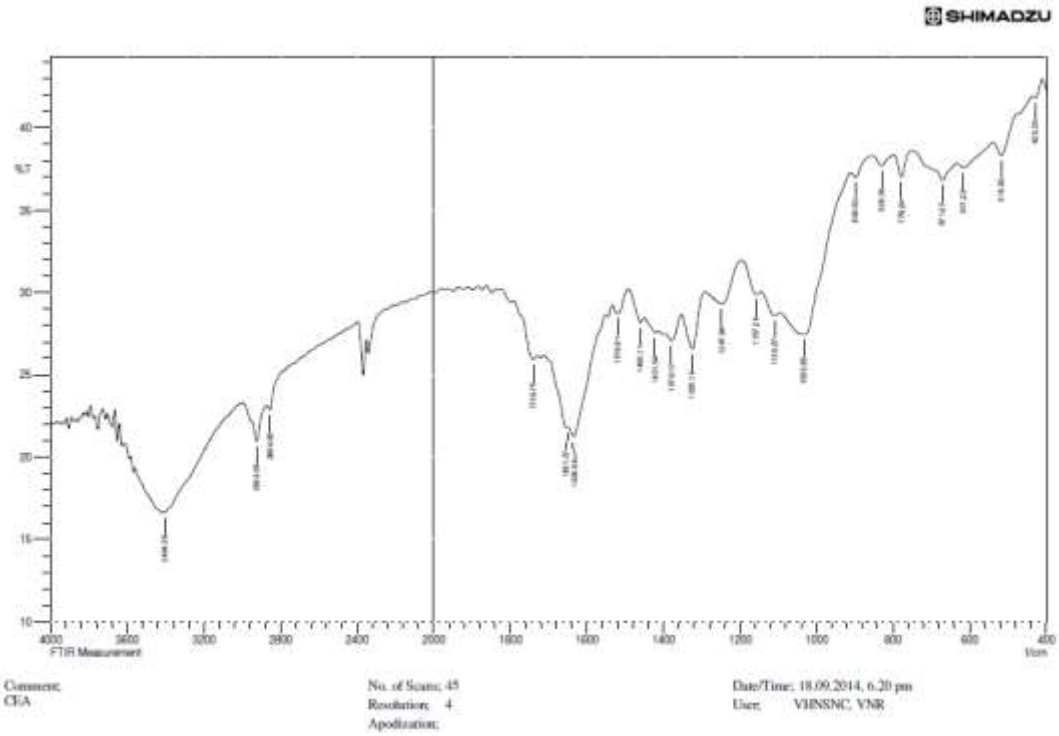

Fig 2, FTIR analysis of Cyclohexane extract

FTIR-Spectrum of Cyclohexane extract shows a strong absorption peak at 3404.36 cm^{-1} which shows strong absorbency for primary and secondary amines (Table 3). The major functional groups includes alkanes, primary amines, nitro compounds, aromatics, carboxylic acids and alkyl halides in the wave number 2924.09& 2854.65, 1635.64&898.39, 1519.91,1421.54,1109.07 and 779.24respectively (Fig.2)

4. CONCLUSION

This study reveals the fact that the potent antimicrobial plant metabolites in Cyclohexane extract of *Achyranthes aspera* had a promising effect for treating the Chronic Wounds. Further study is needed to elucidate the structural properties of the plant metabolites.

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