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RESEARCH ARTICLE

EVALUATION OF *HYPERICUM WIGHTIANUM* WALL. EX WIGHT AND ARN EXTRACTS FOR ANTIBACTERIAL ACTIVITY AGAINST HUMAN PATHOGENS

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ABSTRACT

Aqueous (AEHW) and Methanol (MEHW) extracts of *Hypericum wightianum* was prepared in different dilutions and screened for antibacterial activity against 4 gram negative and 4 gram positive pathogens. The antibacterial activity of extracts (100, 50, 25 and 12.5%) was evaluated using a standard well assay method. The MEHW showed good antibacterial activity comparing to that of AEHW and MEHW also exhibited higher activity upon gram positive pathogens than gram negative pathogens tested.

INTRODUCTION

The use of plants in the management and treatment of diseases started with life. In more recent years, with considerable research, it has been discovered that many plants possess medicinal values. In many parts of the world, most especially in developing countries, there is a major dependence on the use of traditional medicine to treat variety of diseases. For a long period of time, plants have been a valuable source of natural products for maintaining human health (Daniyan and Abalaka 2012). Traditional knowledge of medicinal plants and their use by indigenous culture are not only useful for conservation of cultural traditions and biodiversity but also for community healthcare and drug development now and in the future. Worldwide increase in resistance to antibiotics has prompted scientists and researchers to seek for other possible potential antimicrobials. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. The increasing failure of chemotherapeutic and antibiotic resistance exhibited by pathogenic microbial agents has led to the screening of several medicinal plants for their potential antimicrobial activity. Due to this search, plants have been seen as a good source of antimicrobial agents (Amalu Paul *et al.,* 2014). Many countries from the developing world are still dependent on medicinal plants for treating the sick among them. Globally, the last two decades has witnessed an unprecedented increase of drug resistance by pathogenic microorganisms as well as the appearance of undesirable side effect of certain antibiotics (Akunyilli *et al.,* 1991). Other limitations of modern chemotherapeutic drugs are their high cost and non-availability, especially in rural areas. As a consequence, it is necessary to search for new organic molecules with antimicrobial activity, which in addition could be potential sources for starting materials for the semi-synthesis of new drugs (Anegbeh *et al.,* 2006).

In Ayurveda, Homoeopathy and Unani physicians utilize numerous species of medicinal plants that found their way a long time ago into the *Hindu Materia Medica*. Screenings have been conducted on well known species of plants used in traditional medicines and most plants have shown antibacterial activity (Arunkumar and Muthuselvam 2009). More than a hundred species of therapeutically important higher plants are listed and described in ancient Indian treatise to have the antimicrobial activity. Efforts are thus directed to identify the plant products which have broad spectrum of antimicrobial property with no ill effects (Vasantha *et al.,* 2012). Among the various plants screened for this purpose, the genus *Hypericum*, family Guttiferae, contains about 475 species, occurring world-wide (Robson 2003) has attracted attention with reference to the presence of potential phytochemicals of various applications (Schempp *et al.,* 1999; Fariñas *et al.,* 2008; El-Seedi *et al.,* 2003; Hong *et al.,* 2004; Rath *et al.,* 1996; Tao and Wu 2004; Zofou *et al.,* 2011; Abreu *et al.,* 2004; Iinuma *et al.,* 1995; Gunatilanka *et al.,* 1982; Guo *et al.,* 2005; Chae

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et al., 2006). Considering all these in mind, the present study is concentrated on *Hypericum wightianum* for its antibacterial activity against 4 gram negative and 4 gram positive pathogens using aqueous and methanol extracts of the aerial parts of the *Hypericum wightianum.*

MATERIALS AND METHODS

Plant material and extraction

The aerial parts of *H. wightianum* (Fig.1 & 2) were collected from Kodaikanal hills in the month of June 2013. The plant was authenticated by Dr. M.Palanisamy, Scientis 'C' In-charge, Botanical Survey of India, Coimbatore, Tamil nadu, India. A voucher specimen was preserved in our laboratory for future reference (voucher No. BSI/SRC/5/23/2013-14/Tech.850, Dt.21 August 2013). The plant material was shade dried, pulverized and extracted (500g) with methanol and distilled water at room temperature for 72 hrs. The extract was filtered and concentrated to dryness under reduced pressure and controlled temperature (40 – 50°C) in a rotary evaporator. Both the extracts were dark greenish brown solid weighing MEHW 89.30 gr. (yield, 17.86) & AEHW 51.25 gr. (yield, 10.23%) and were preserved in a vacuum desiccator at 4°C until further use. Different concentrations (100%, 50%, 25%, and 12.5%) of extracts were prepared by dissolving extracts in dimethylsulfoxide (DMSO).

Morphology of *Hypericum wightianum*

Fig. 1.

Fig. 2.

Test Organisms

The following eight strains of pathogenic bacteria were used in all antimicrobial screening:

Gram-positive bacteria	Gram-negative bacteria
Staphylococcus aureus (GP1)	*Escherichia coli* (GN1)
Staphylococcus haemolyticus (GP2)	*Salmonella enteric* (GN2)
Streptococcus agalactiae (UN)	*Aeromonas sobria* (GN3)
Listeria monocytogenes (GP4)	*Klebsiella oxytoca* (GN4)

Bacterial susceptibility test

The susceptibility of the test bacteria to plant extracts was determined using a well diffusion assay on Mueller-Hinton agar plates, following the method described in NCCLS manual (2003). Diluted bacterial cultures were adjusted to 0.5 McFarland turbidity (1-2 x 106 CFU mL-1) and spread evenly over the entire surface of the agar plates using a sterile cotton swab. The plates were allowed to air-dry for approximately 10 min before wells (6 mm holes) were cut into the agar using sterile plastic straws. Different concentrations of extracts of plant were filled in individual well (15µl). A control plate for different solvents (Distilled water, Methanol and DMSO) were also prepared. The plates were incubated at 37°C for 24hrs period. For each microorganism tested, zones of inhibition of growth were examined, and the diameter of each zone was measured and recorded.

RESULTS

Table 1 shows control and plant extracts and their activities against the various organisms. In the present investigation extracts of *Hypericum wightianum* screened, MEHW showed good antibacterial activity compared to AEHW. The control groups Distilled water, methanol and DMSO did not exhibit any inhibitory action against any bacteria. The AEHW showed some activity in higher concentration only. But the MEHW exhibited strong antibacterial activity in gram +ve and gram –ve bacteria. MEHW showed good activity upon gram +ve comparing with gram –ve bacteria. Strong activity exhibited by MEHW is upon *Staphylococcus aureus* and *Listeria monocytogenes* (Fig.3 & Fig.4). Relatively similar level of activity showed in other two gram +ve bacteria also (*Staphylococcus haemolyticus* and *Streptococcus agalactiae*).

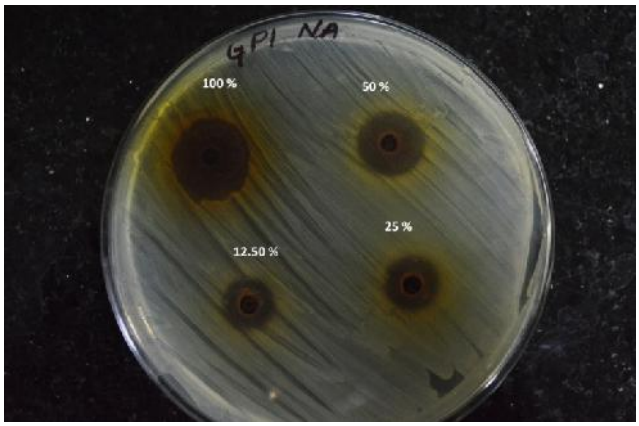

Fig.3. Methanol (Staphylococcus aureus GP1)

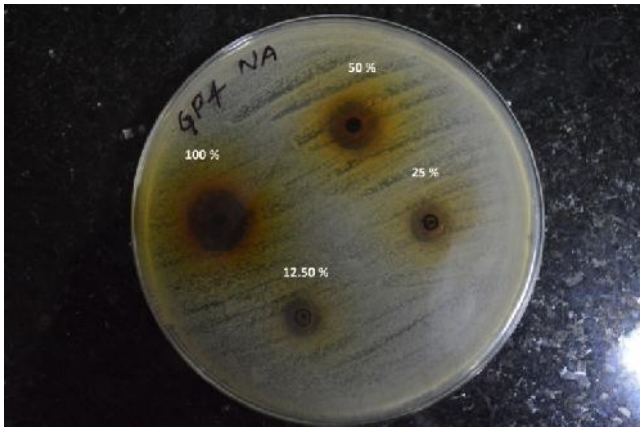

Fig.4. Methanol (*Listeria monocytogenes* GP4)

Table 1. Antimicrobial test results

1. Aqueous Extracts

S.No.	Name of the pathogens	Zone of Inhibition (mm) After 24h incubation			
		100%	50%	25%	12.5%
	Gram +ve bacteria				
1.	*Staphylococcus aureus* GP1	0	0	0	0
2.	*Staphylococcus haemolyticus* GP2	8	0	0	0
3.	*Streptococcus agalactiae* UN	8	6	5	4
4.	*Listeria monocytogenes* GP4	10	6	0	0
	Gram -ve bacteria				
1.	*Escherichia coli* GN1	12	10	8	0
2.	*Salmonella enterica* GN2	0	0	0	0
3.	*Aeromonas sobria* GN3	10	5	0	0
4.	*Klebsiella oxytoca* GN4	11	0	0	0

2. Methanol Extract

S. No.	Name of the pathogens	Zone of Inhibition (mm) After 24h incubation			
		100%	50%	25%	12.5%
	Gram +ve bacteria				
1.	*Staphylococcus aureus* GP1	17	14	11	9
2.	*Staphylococcus haemolyticus* GP2	10	6	-	-
3.	*Streptococcus agalactiae* UN	13	11	10	9
4.	*Listeria monocytogenes* GP4	14	11	9	8
	Gram -ve bacteria				
1.	*Escherichia coli* GN1	5	-	-	-
2.	*Salmonella enterica* GN2	10	9	7	5
3.	*Aeromonas sobria* GN3	11	10	9	7
4.	*Klebsiella oxytoca* GN4	12	10	8	7

Conclusion

The type and level of biological activity exhibited by any plant material depends on many factors, including the plant part, geographical source, soil conditions, harvest time, moisture content, drying method, storage conditions, and post-harvest processing. For example, the relatively high temperatures that can be generated during tissue grinding can denature chemical constituents and the extraction solvent, time period, and temperature can affect the level and composition of secondary metabolites extracted from plant tissues. In general, the antibacterial activity of plant extracts appears to be inhibitorier to Gram-positive bacteria than Gram-negative bacteria. It should be remembered that penicillin and some of the other prominent antibiotic agents of fungal origin are also rather selective in their inhibitory action, most of them being inhibitory to Gram-positive bacteria. Unlike Gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram-negative bacteria (NCCLS 2003). The outer lipopolysaccharide layer hinders access of most compounds to the peptidoglycan layer of the cell wall. This explains the resistance of Gram-negative strains to the lytic action of most extracts exhibiting activity. The comparatively lower level results obtained against Gram-negative bacteria were not unexpected since this class of bacteria is usually more resistant than Gram-positive bacteria (Kirtikar and Basu 1968). The antimicrobial extracts of tested plant can be assumed to be useful to the producing plant in warding off infectious diseases and there is therefore a compelling reason to suppose that anti-infective agents could be active against human pathogens.

A number of studies have voiced the necessity of developing alternative antimicrobial drugs (Turnbull *et al.*, 1991; Poole 2002). Plant antimicrobials would appear to be an excellent choice (Sibanda and Okoh 2007; Mahady 2005). This study revealed that *Hypericum wightianum* used in this study producing strong antimicrobials, especially MEHW, and may offer prospective new treatments for bacterial infections. The benefit of antimicrobial properties from *Hypericum wightianum* can only be achieved, however, by using a specific solvent and solvent concentration in extracting the plant materials. The study of chemical profiles of extracts obtained with different solvents warrants future research to determine the active extract constituents.

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