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Full Length Research paper

Assessment of *Commiphora wightii* (Arn.) Bhandari (Guggul) as potential source for antibacterial agent

Pankaj Goyal, Abhishek Chauhan and P. Kaushik

Department of Botany and Microbiology, Gurukul Kangri University, Hardwar Uttarakhand - 249 404, India

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There is a great need to discover novel antibiotics due to wide-spread emergence of resistance among pathogenic bacteria against available antibiotics. The current study deals to access the susceptibilities of some clinically significant bacteria against various crude extracts of *Commiphora wightii* gum resin. Agar well diffusion assay was the key process to evaluate the antibacterial potential of various crude extracts of the plant. Active crude extracts were further subjected to determine MIC against susceptible bacteria. The study indicated that antibacterial activity was found to be dependent on the type of extract and the organism evaluated. Ethanol extract was found to have comparatively higher activity than other organic and aqueous extracts. Gram-positive bacteria showed competent but variable susceptibilities to all the tested extracts. MIC data showed hopeful results as some of the extracts exhibited significant inhibitions of bacteria even at low concentrations. Phytochemical analysis of the active extract reveals the presence of certain metabolites. *Commiphora wightii* gum resin is shown to have promising antibacterial activity that could be very useful in the discovery of novel antibiotic.

Keywords: Antibacterial activity, *Commiphora wightii* (Arn.) Bhandari, guggul, minimum inhibitory concentration**.**

INTRODUCTION

Antibiotic chemotherapy has been one of the most important medical achievements of the twentieth century. This therapy is widely practiced for the treatment of various microbiological infections. In recent years, the prevalence of antimicrobial resistance among key microbial pathogens such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella*, *Haemophilus*, *Neisseria*, *Moraxella* and *Enterococcus faecalis* is increasing at an alarming rate worldwide (Cohen, 1992; Gold and Moellering, 1996; Kaushik and Goyal, 2008a). The outcome is that many antibiotics can no longer be used for the treatment of infections caused by such organisms and the threat to the usage of other drugs is steadily increasing (Courvalin, 1996; WHO, 2000). A feasible way to combat the problem of microbial resistance is the development of new antibacterial agents for substitution with ineffective ones.

Natural products have played an important role throughout the world in treating and preventing human diseases (Newman et al., 2000; Chin et al., 2006; Kaushik et al., 2008). Plant-based products are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999). They may become the base for the development of a medicine, a natural blueprint for the development of new drugs (Ahmad et al., 1998; Goyal et al., 2008). An illustrated description of medicinal plants have been mentioned in Ayurveda, which literally means 'the science of life' *i.e*. *Ayur* (life) and *Veda* (knowledge). Many herbs used by Ayurvedic practitioners show promising results and could be appropriate for larger randomized trials. It is presumed that the broad-spectrum effectiveness of these spices may provide a suitable basis for new antimicrobial therapies (Kaushik, 2003; Kaushik and Goyal, 2008b).

Commiphora wightii (Arn.) Bhandari is an important member of family Burseraceae. The plant is a shrub or small tree, having a height of 4-6 foot, with thin papery bark. The plant grows wild in northern India, where it has been reported to be found in arid and semi-arid climates and unfertile soils of Rajasthan, Karnataka and Gujarat. According to Ayurveda, there are five types of *Guggulu* namely; *Krishnan* (black), *Peet varn* (yellow), *Neel* (blue),

*Corresponding author email: pankaj.goyals@gmail.com; Tel. Number: +91-999 000 7377

Kapish (light brown) and *Rakt* (blood red); among which only first two are suitable for human consumption.The *Atharvaveda*; one of the four well known Holy Scriptures (*Vedas*) of the Hindus, is one of the earliest references to the medicinal and therapeutic properties of guggul. *Chikitsa Sthanam* of *Charaka Samhita* also describes various medicinal properties of *Guggulu*.

Oleogum resin is the economically viable part of the plant. It is excreted by specialized cells or ducts in plants, especially from stem-bark. As per constituents, it has 6.9% moisture, 0.6% volatile oil, resin 61%, gum 29.6%, and insoluble substances 3.2%.

The current study dealt with the evaluation of gum-resin part of the Guggul for possible antibacterial activity followed by determination of minimum inhibitory concentration and phytochemical analysis of the active crude extracts.

MATERIALS AND METHODS

Plant material

Gum resin of Commiphora wightii (Arn.) Bhandari was collected locally in late October of 2007. The plant was taken to the laboratory and was authenticated by Prof. P. Kaushik at Department of Microbiology, Gurukul Kangri University, Hardwar (India).

Extract preparation

Gum- resin of guggul were extensively washed under running tap water for removal of dust particles and epiphytic hosts normally found on the surface, followed by washing with sterilized distilled water. They were further air- dried on filter paper at room temperature and then powdered with the help of sterilized pestle and mortar under aseptic condition. Dry powder was further extracted by using following methodologies.

Aqueous Extraction

Air-dried powder (10 g) of the respective plant part was mixed well in 100 ml sterilized distilled water and kept at room temperature for 24 h on an orbital shaker with 150 rpm. The solution was further filtered using muslin cloth. The filtrate was centrifuged at 5000 rpm for 15 minutes. The supernatant thus obtained was filtered through Whattman's Filter No. 1 under strict aseptic conditions and the filtrate was collected in a pre-weighed sterilized test tube. Aqueous extracts were prepared in final concentration of 100 mg ml^{-1}. Test tubes were cotton plugged and stored in refrigerator at 4°C until further used.

Organic Solvent Extraction

Air-dried powder (10 g) of the respective plant part was thoroughly mixed with 100 ml organic solvent (*viz*.; ethanol, methanol, ethyl acetate and hexane). The mixture was placed at room temperature for 24 h on orbital shaker at 150 rpm. Solution was filtered through muslin cloth and then re-filtered by passing through Whattman's Filter No. 1. The filtrate thus obtained was concentrated by complete evaporation of solvent at room temperature (25°C) to yield the pure extracts. Stock solutions of crude extracts from each of the organic solvents were prepared by mixing well the appropriate amount of dried extracts with the respective solvent to obtained a final concentration of 100 mg ml^{-1}. Each solution was stored in refrigerator 4°C after collecting in sterilized bottles until further used.

Bacterial strains

A total of six bacterial strains including both Gram-negative and Gram-positive bacteria (*Escherichia coli* MTCC-739, *Salmonella typhi* MTCC-531, *Bacillus cereus* MTCC-430, *Bacillus subtilis* MTCC-736, *Streptococcus pyogenes* MTCC-442, and *Staphylococcus aureus* MTCC-740) were selected to assess susceptibility patterns against the extracts prepared in the present study. The bacterial cultures were maintained in nutrient agar slants at 37°C. Each of the microorganisms was reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37°C.

Antibacterial Susceptibility Assay

Extracts obtained by various processes were evaluated for their potential antibacterial activities by the standard agar well diffusion assay (Perez et al., 1990). All extracts were sterilized by sterile membrane syringe filter (pore size 0.45 µm, (Pall Life Sciences, U.K.) . Petri dishes (100 mm) containing 18 ml of Mueller Hinton Agar (MHA) were seeded with approximately 100µl inoculum of bacterial strain (inoculum size was adjusted so as to deliver a final inoculum of approximately 10^8 CFU/ml) and allowed to solidify. Wells of 6 mm diameter were cut into solidified agar media using a sterilized cup-borer. 100µl of each extract was poured in the respective well and the plates were incubated at 37°C overnight. The experiment was performed in triplicate under strict aseptic conditions to ensure consistency of all findings. The antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by each extract at the end of incubation period.

Sterilized distilled water and other solvents used in preparation of extracts were used as negative controls. Tetracycline (5 g/ml) was used as a standard antibiotic (*i.e*. positive control) in the present study for a comparative analysis with the effectiveness of various plant extracts against selected microflora.

Assessment of Minimum Inhibitory Concentration

Active extracts obtained by agar well diffusion assay were further subjected to determine the minimum inhibitory concentration (MIC) required for the bacteriostatic effects by standard two-fold broth microdilution methodology (NCCLS, 1997). A stock solution of each active extract was serially diluted in 96-wells microtiter plate with Mueller Hinton broth to obtain a concentration ranging from 8.0 µg/ml to 4096 µg/ml. A standardized inoculum for each bacterial strain was prepared so as to give an inoculum size of approximately 5×10^5 CFU/ml in each well. Microtiter plates were then kept at 37°C for an overnight incubation. Following incubation, the MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial strain using reflective viewer.

Phytochemical Analysis

Active crude extract prepared from resin of *Commiphora wightii* (Arn.) Bhandari was further analyzed for phytochemical constituents using standard methods (Stahl, 1969; Harborne, 1973).

Table 1. *In vitro* antibacterial activity of aqueous and organic extracts of *commiphora wightii* gum-resin

Type of Extract			Zone of Inhibition* (in mm diameter)					
			Gram-negative Bacteria			Gram-positive Bacteria		
			Escherichia coli	***Salmonella typhi***	***Streptococcus pyogenes***	***Staphylococcus aureus***	***Bacillus subtilis***	***Bacillus cereus***
Organic Extract		Ethanol	08.16±1.26	NI	NI	14.50±0.86	08.83±0.57	NI
		Methanol	14.83±0.76	NI	NI	19.67±0.76	14.50±1.73	17.50±1.00
		Ethyl Acetate	NI	NI	NI	15.00±1.73	11.67±2.52	08.16±0.76
		Hexane	NI	NI	NI	09.33±0.58	NI	NI
	Aqueous Extract		NI	NI	NI	13.16±1.04	NI	NI
Control	**Positive**	Tetracycline^{+}	29.50±0.50	25.83±1.61	29.83±1.89	32.50±1.50	34.17±1.76	32.16±1.04
	Negative	Ethanol	NI	NI	NI	NI	NI	NI
		Methanol	NI	NI	NI	NI	NI	NI
		Ethyl Acetate	NI	NI	NI	NI	NI	NI
		Hexane	NI	NI	NI	NI	NI	NI

*Values of the observed zone of inhibition (in mm diameter) including the diameter of well (6 mm) after 24 hours incubation against different bacterial species when subjected to different extracts in agar well diffusion assay. Assay was performed in triplicate and results are the mean of three values ± Standard Deviation. In each well, the sample size was 100 µl. Inhibition observed in extracts due to solvent were assessed through negative controls. 'NI'-No Inhibition Zone was observed. $^{+}$Tetracycline (5 g ml^{-1}) was used as standard antibiotic.

RESULTS

Agar well diffusion assay

Antibacterial activity of gum resin extracts of *Commiphora wightii* has been demonstrated in Table 1. This shrub is an important medicinal plant having a number of therapeutic properties and it belongs to family Burseraceae. Data indicated that methanol extract was found to have maximum inhibitory power against almost all the bacteria tested (zone of inhibition ranges from 14.83 mm to 19.67 mm) except *Salmonella typhi* and *Streptococcus pyogenes*. These two bacteria were found resistant to all the extracts evaluated. Ethanol and ethyl acetate extracts were found inhibitory but comparatively lower than that of methanol extracts. Hexane and aqueous extracts were found to be effective only against *Staphylococcus aureus* (zone of inhibition 9.33 mm and 13.16 mm, respectively). The maximum susceptibility was shown by *Staphylococcus aureus* which was inhibited almost all the gum resin extracts followed by *Bacillus subtilis* (inhibited by methanol, ethanol and ethyl acetate extracts), *Bacillus cereus* (inhibited by methanol and ethyl acetate extract) and *Escherichia coli* (inhibited by ethanol and methanol extracts).

Minimum inhibitory concentrations

Active crude extracts (with zone of inhibition 12 mm) of guggul were further evaluated by microbroth dilution assay to determine minimum inhibitory concentrations (Table 2). Methanol extract was found significant inhibitory against *Staphylococcus aureus* with MIC 512 µg ml^{-1}. The same extract was found active against *Bacillus cereus* and *Bacillus subtilis* at 1024 µg ml^{-1} and 2048 µg ml^{-1}, respectively. MIC of this extract against *Escherichia coli* was not observed (*i.e*. >4096 µg ml^{-1}). *Staphylococcus aureus* was also inhibited by other extracts *viz*., ethyl acetate

Table 2. Minimum inhibitory concentration of active crude extracts of *commiphora wightii* gum-resin

Type of Active Crude Extract	Test Microorganism	Concentration of Extracts* (in g ml^{-1})										MIC (in g ml^{-1})
		4096	2048	1024	512	256	128	64	32	16	8	
Ethanol	*Staphylococcus aureus*	-	+	+	+	+	+	+	+	+	+	4096
Methanol	*Staphylococcus aureus*	-	-	-	-	+	+	+	+	+	+	512
Methanol	*Bacillus cereus*	-	-	-	+	+	+	+	+	+	+	1024
Methanol	*Bacillus subtilis*	-	-	+	+	+	+	+	+	+	+	2048
Methanol	*Escherichia coli*	+	+	+	+	+	+	+	+	+	+	ND
Ethyl Acetate	*Staphylococcus aureus*	-	-	+	+	+	+	+	+	+	+	2048
Ethyl Acetate	*Bacillus subtilis*	+	+	+	+	+	+	+	+	+	+	ND
Aqueous	*Staphylococcus aureus*	+	+	+	+	+	+	+	+	+	+	ND

*****Different concentrations of active crude extracts evaluated in 96-well microtiter plate using Microbroth Dilution Assay as recommended by NCCLS. All values are expressed in g ml^{-1}; (-) represents 'No Growth Observed'; (+) represents 'Growth Observed'; ND = Not Detectable.

and ethanol at the concentration of 2048 µg ml^{-1} and 4096 µg ml^{-1}, respectively; while the aqueous extract didn't show inhibition in the entire range of extract's dilutions. *Bacillus subtilis* was not inhibited by ethyl acetate extract even at concentration more than 4096 µg ml^{-1}.

Phytochemistry

Phytochemical evaluation revealed the presence of alkaloids, glycosides, steroids, terpenoids and flavonoids in methanol extract of guggul.

DISCUSSION

Commiphora wightii (Arn.) Bhandari **(***Guggulu*) is *vata* and *kapha* suppressant and is widely used in the diseases caused by *vata*. It is a good pain reliever and also acts as an anti-inflammation. It promotes wound healing and is very effective as vermicidal, it strengthens the nervous system, promotes digestion, good for liver and other medical cases ((Kaushik and Singh, 2004). A small controlled trial compared oral gugulipid against tetracycline for the treatment of acne and other skin related problems also reported a similar results. Guggul gum has been employed as a traditional remedy in the practice of various Ayurvedic medicines. Purported benefits of guggul gum included relief from epilepsy, ulcers, obesity and rheumatoid arthritis (Jachak and Saklani, 2007).

Herbal extracts from *Commiphora mukul* (guggul) have been widely used in Asia as cholesterol-lowering agents and their popularity is also increasing in the United States (Jachak and Saklani, 2007). Recently, guggulsterones, the purported bioactive compounds of guggul, have been shown to be potent antagonists of two nuclear hormone receptors involved in cholesterol metabolism, establishing a plausible mechanism of action for the hypolipidemic effects of these extracts (Jachak and Saklani, 2007).

Hammer et al. (1999) evaluated the inhibition of Gram-positive bacteria by the essential oil of *Commiphora myrrha.* Romero et al. (2005) reported the similar results while studying the antibacterial activity of *Commiphora molmol*. The ethyl acetate extract of aerial parts of *Commiphora opobalsamum* L. was found moderately active against *Staphylococcus aureus* as studied by Abbas et al. (2007), which is in agreement with this present finding.

Our findings support the traditional medicinal use of this plant and its future aspects in developing novel antimicrobials. Guggul can potentially be used in the treatment of various inf-

ectious diseases caused by microorganisms that are showing resistance to currently available antibiotics. Furthermore, active plant extracts can be subjected to various pharmacological evaluations by several methods such as GC-MS, NMR (nuclear magnetic resonance), Mass Spectrometry etc. for the isolation of the therapeutic antimicrobials.

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