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Original article

In-vitro anti-microbial activity of methanolic extract of Atropa belladonna using the agar well diffusion method

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ABSTRACT

Traditional plants have many medicinal values including the treatment of different infectious diseases. In the assessment of conventional claims, scientific research is foremost. For this reason, the methanolic extract of Atropa Belladonna were evaluated at different concentration for antimicrobial activity against medically important bacteria viz. Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia. The in vitro antimicrobial activity was performed by agar well diffusion method. The methanolic extract of Atropa belladonna exhibit considerable degree of antimicrobial activity against the tested gram negative and gram-positive bacterial strains.

Keywords: antimicrobial activity, Atropa Belladonna

INTRODUCTION

A great number of infectious diseases are used to be treated with herbal remedies in the history of humankind. The discovery and appraisal of medicinal plants throughout different areas of the world is important in all the healthcare sectors, in establishment of advanced directions towards the cultivation and propagation of various alternative medicinal crops that offer far better socioeconomic benefits. Generally the traditional Medicinal plants can play a paramount role in alleviating several ailments worldwide.

Traditionally employed medicinal plants produce a significant variety of compounds of known therapeutic properties [1]. Albeit those substances that can either kill the pathogens or inhibit their growth as well as have little or no toxicity to the host cells are contemplated candidates for the innovation of the antimicrobial drugs. Moreover it is anticipated that extracts of plants shows target sites other than those utilized by antibiotics will be active against the drug-resistant microorganism. Yet, very little details are available on such activities of medicinal plants.[2] Although the management of bacterial infections have remarkably been effective since the discovery of the different antibacterial drugs. Nevertheless some of the pathogens have rapidly become resistant to several of the first discovered effective drugs. The development of drug resistance as well as manifestation of untoward effects of certain antibiotics. [3] has led to the investigation of new antibacterial agents particularly from medicinal plants. Furthermore, a number of these traditional plants of medicinal claims are generally have been used by people as folk medicine for several decades.[4] One of the most extensively studied horticultural medicinal plant, the *Atropa belladonna*, associated with the *Solanaceae* family is known to rich in tropane alkaloids, mainly used as antimicrobial compounds in different pharmaceutical drugs [5].Usually, plants that belong to the family solanaceae are abundant in the tropane alkaloids, which are found in most parts, predominantly in the flowering tops and leaves. The present study has conducted to screen the antimicrobial activity of Atropa belladonna methanolic extract against some common bacterial species.

MATERIALS AND METHODS

Plant Material: Fresh plant and its parts were collected arbitrarily from the Multan region of Pakistan. Whole plant except Flowering tops or berries and roots were used to make the extract.

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The taxonomic identities of these plants were confirmed by the Department of pharmacy, Hamdard University. Plant material then washed with clean water, air dried and then converted to fine powder and stored in airtight container.

Preparation of plant extracts: Plant extracts was prepared as described by [6] with little variation. Particularly the alcoholic extracts were tested, as alcohol was found to be a better solvent for extraction of substances that are antimicrobial active as compared to water and hexane [7]. Shortly, 100 g of powdered plant material were soaked in 250 ml of 90% methanol for almost four days. The mixture was stirred carefully at every 18 hours by using a sterile glass rod. In the last part of extraction, the extract was passed via Whatman filter paper (Whatman Ltd., England). Furthermore, the filtrate then air dried for few days then become concentrated at room temperature of 30°C and stored in refrigerator for the additional use. Eventually the percentage yield of crude extract of plant sample was resolute in terms of milligrams.

Microorganisms: Antimicrobial activity was tested in vitro for methanolic extract of the renowned traditional healer plant named Atropa belladonna. Microorganisms were obtained from the Local nearby Certified Biochemistry Laboratory, Karachi, Pakistan. The pathogenic Microbes were maintained at 4°C on prepared nutrient agar slants. Amongst three pathogenic microorganisms examined, two Gram negative bacteria were Escherichia coli and Klebsiella pneumonia while a Gram postive bacterium was Staphylococcus aureus.

Antimicrobial Activity: The antimicrobial activity testing was performed by agar well diffusion method [8] particularly for solvent extract. The suspensions of the microorganisms were produced by suspending of pure culture nearly a loop full in 10 ml of distilled sterile water. Furthermore 1 ml of pure culture of bacterial suspension was separately mixed with 10 ml of sterile molten Nutrient Agar and pour medium in unlike sterile petri dishes (already labeled with bacterial name and compound under study).

Moreover the media of petri dishes were divided into equivalent parts after solidification and similar holes were made in the center of each part by the help of a cork borer. Now two or three drops of the premeasured methanolic extract of various concentrations were placed into the individual holes of different petri dishes which were already marked and then incubated at 37°C in incubator for 24 hours for the bacteria. Subsequently, growth of microorganism was determined by measuring the diameter of the zones of inhibition which is measured by the help of digital vernier calipers. Controls were maintained for each bacterial strain where discs of standard medicine were used instead of the plant extract. Thus, the result was obtained by measuring the zone diameter. Finally, the results were compared with the standard antimicrobial discs.

RESULTS AND DISCUSSIONS

This aforementioned study has been carried out to primarily investigate the antimicrobial activity of methanolic extract at various concentration of the well-known medicinal plant Atropa belladonna against the gram positive and gram negative bacteria. The antibacterial activity of the crude plant extracts against the specific pathogenic bacterial strains was evaluated by the presence or the absence of inhibition zones and MIC (minimum inhibitory concentration) values. The most liable bacteria to the tested plant extract were gram positive S. aureus that showed the highest zone of inhibition i.e. 8.08 mm, moreover S.aureus is responsible to cause soft tissue infection and food poisoning. On the other hand, the least susceptible bacteria were gram negative K. pneumoniae that showed the lowest zone of inhibition i.e. 5.41 mm, this bacteria is predominantly responsible to cause Pneumonia, other respiratory and Urinary tract infections. While gram negative E.coli showed the moderate susceptibility.

Gram negative pathogenic E.coli is responsible to cause gastroenteritis, neonatal meningitis and urinary tract infections. Such infections can be treated with standard drug includes levofloxacillin, cefepime, Gentamicin and piperacillin/tazobactam respectively. But not all strains of bacteria species can be successfully treated by these drugs. Various workers have already shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative bacteria [9]. These differences may be attributed to fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure [10].

This restrains the penetration of active drug fraction. It is contemplated that differences in the observed values may result from the doses used in the experiment as well as the choice of solvent for the preparation of extracts is responsible for these variations since consecutive isolation of medicinal compounds from plants is greatly dependent on the type of solvent used in the process of extraction. Additionally, microorganisms show unpredictable sensitivity to chemical substances related to different resistance levels between strains [11] .

The crude methanolic extract of the plant Atropa belladonna investigated has showed the noteworthy activity in dose dependant manner. This quest can pave the way for the further pharmacological investigation for this plant and promising the beginning of more research and scientific evaluation considering the traditional medicinal claims.

Table 1: Zone of inhibition (mm) of Plant extract on different concentrations

Microorganisms	Atropa Belladonna (methanolic extract)			
	10mg/ml	100mg/ml	500mg/ml	1000mg/ml
Escherichia coli	7.12	6.31	5.98	5.66
	7.33	5.91	5.87	5.10
	6.97	7.53	6.11	6.35
Average values	7.14	6.58333	5.98667	5.70333
Staphylococcus aureus	8.16	7.79	6.45	6.15
	7.98	8.12	6.11	5.79
	8.11	7.55	7.11	6.79
Average values	8.08333	7.82	6.55667	6.24333
Klebsiella pneumoniae	6.67	5.87	5.55	5.56
	6.12	5.99	5.42	5.23
	6.01	6.61	5.95	5.45
Average values	6.26667	6.15667	5.64	5.41333

Table 2: Zone of inhibition (in mm) by Control (standard medicines)

Microorganisms	Levofloxacin 5microgram/disc	Gentamicin 10microgram/disc	Piperacillin 110microgram/disc
Escherichia coli	11.01	6.85	8.14
	11.52	7.12	8.55
	11.52	6.97	8.57
Average values	11.3467	6.98	8.42
Staphylococcus aureus	7.58	4.93	9.01
	8.23	5.53	8.96
Average values	8.56	5.56	9.15
	8.21333	5.37667	9.04
Klebsiella pneumoniae	2.12	5.23	9.11
	2.53	6.11	8.99
Average values	3.01	5.79	9.76
	2.55333	5.71	9.43333

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