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ORIGINAL RESEARCH ARTICLE

In vitro Antibacterial study of two commonly used medicinal plants in Ayurveda: Neem (*Azadirachta indica* L.) and Tulsi (*Ocimum sanctum* L.)

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ABSTRACT

With the widespread development of resistance of microbial pathogens against currently available modern antibiotics, medical science is now making efforts to discover novel antibiotics. Traditional herbs like Neem (*Azadirachta indica* L.) and Tulsi (*Ocimum sanctum* L.) have been proved to be a better alternative. In the present study an effort has been made to access the susceptibilities from aqueous and ethanol extracts of Neem (leaves and bark) and Tulsi (leaves) against some clinically significant bacterial species. Antimicrobial activity has been done by disc diffusion method against two Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram negative bacteria (*Klebsiella pneumoniae* and *Escherichia coli*). The ethanolic extract of Neem bark showed more significant activity at 250 µg concentrations than Neem leaves at all the studied concentrations. *E. coli* was highly susceptible to ethanol extract of Tulsi leaves at 250 µg concentration. Most bacterial strains were resistant at lowest concentrations (5 µg) of various extracts of both plants. The study indicates that Neem bark was found to possess more significant antibacterial activity than Neem leaves and Tulsi leaves.

Key words: *Azadarichta indica*, *Ocimum sanctum*, Antibacterial activity, Disc diffusion, Zone of Inhibition, Bark, Leaves.

INTRODUCTION

Natural herbs and their varied extracts have been used globally in therapeutic since antiquity [1]. In developing countries majority of the population still exploits traditional folk medicine derived from plant resources [2,3]. Medicinal use of herbal preparations is first mentioned in ancient Hindu texts like Vedas and these herbs are an important part of 'medicinal science of Indian culture – Ayurveda [4].

In current epoch quick results are expected; hence haphazard use of synthetic antimicrobial drugs is copious these days which is now resulting in multiple drug resistance [5,6] and evidences of serious adverse effects are noted in various studies [7]. Therefore natural herbs are gaining importance in overcoming this problem [8], as the traditional herbs are found to be more economical and having lesser side effects than synthetic drugs [9, 10].

Neem (*Azadirachta indica*) belonging to family Meliaceae and Tulsi (*Ocimum sanctum* L.) belonging to the family Lamiaceae are used for medicinal purpose since centuries in India [11,12,13]. Twig of its stem is widely used for chewing (brushing) in rural areas since time immemorial and it is now proved to have antiplaque and related antibacterial properties [14, 15]. Tulsi leaves are reported to exhibit insecticidal and antibacterial activities [16]. The crude plant and its extracts are used in various infections and as a cough remedy and expectorant based on the traditional experience [17].

Decoction and oil extracted from Neem leaf is reported to have excellent antiseptic[18] and broad spectrum antibacterial activity action against Gram-negative and Gram-positive microorganisms, including *M. tuberculosis* and Streptomycin resistant strains [19]. From this point of view taking a note of this it was felt to explore comparative antibacterial activities of aqueous and ethanol extracts of Neem bark, Neem leaves and Tulsi leaves against selected microorganisms.

MATERIALS AND METHODS

Procurement and identification of plant materials

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The medicinal plants used for the evaluation of antimicrobial study were Neem (*Azadirachta indica*) and Tulsi (*Ocimum sanctum*)**.** Fresh bark and leaves of Neem and leaves of Tulsi were collected in the month of April, 2011 from the IPGT & RA, GAU campus Jamnagar and identified at Pharmacognosy Laboratory.

Preparation of the plant material

Leaves and barks were shade - dried and converted into coarse powder by grinder and then stored at room temperature and were subjected to the following extraction protocols.

Preparation of the Extracts

Powdered sample were subjected to successive extraction with water, and 50% ethanol by Soxhlet method[20]. The extracts were collected and distilled off on a water bath at atmospheric pressure. Extracts were stored in refrigerator for antimicrobial studies.

Test Microorganism

The microorganisms in the present study were: Gram Positive - *Bacillus subtilis* (BS) (MTCC B2274) and *Staphylococcus aureus* (SA) (MTCC96), Gram Negative - *Klebsiella pneumoniae* (KP) (MTCC B2405) and *Escherichia coli* (EC) (MTCC 739). All the cultures were purchased from microbial type culture collection (MTCC) Chandigarh.

***In vitro* antimicrobial activity**

Determination of Zone of inhibition by Disc diffusion method [21,22]. Muller hinton agar (Hi Media-M173-500G) was prepared in the plates as the media for test organisms. Sterile whatmann No. 1 filter paper was used to prepare discs of diameter about 6 mm. The cultures are diluted to a density equivalent to the 1 % barium sulfate standard to obtain suspension containing approximately (1-2 X 10^8 CFU/ ml). 0.1 ml of each microbial suspension was spread evenly onto the surface of the agar plates using sterile glass spreader. After the inoculation of culture the sterile disc were carefully loaded with 20 µl of different dilution of each extract and placed onto the agar plates. All the plates were incubated at 37 ^{0}C for 48 hours.

Standard Drug Used

Chloramphenicol and ciprofloxacin was used as standard antibiotic against one Gram positive and one Gram negative organism (**Table 4**).

RESULTS AND DISCUSSION

Various biological active constituents are isolated from plants which possess antimicrobial[23], anti-inflammatory and other therapeutic values [24, 25].The phytochemical constituents of Neem and Tulsi have been established in previous studies and these include tannins, alkaloids, carbohydrates, phenols, flavonoids, glycosides etc [26, 27, 28].

The preliminary screening of antimicrobial activity of aqueous and ethanol extracts of *A. indica* leaves against bacterial strains are shown in (**Table 1**). Both the extracts of leaves of *A. indica* expressed antibacterial activity on at least one bacterium. Ethanol extract was the most effective against all the tested bacteria. Aqueous extract did not show any activity at low concentrations on *S. aureus*, *E. coli* and *Klebsiella pneumonia*. At other concentrations also, its activity was found to be weaker in comparison to ethanol extract. On the basis of zone of inhibition, maximum activity was found at the concentration of 250 µg and in this case also ethanol extract showed better results. *S. aureus* and *E. coli* were found to be more susceptible followed by *Klebsiella pneumonia* and *Bacillus subtilis*. Bark also depicted similar results with slight variation in activity (**Table 2**). Aqueous extract was found to be less effective in antimicrobial activity in comparison to ethanol extract. Degree of susceptibility in descending order was maximum for *E. coli* followed by *S. aureus*, *K. pneumonia* and *B. subtilis* at 250 µg concentaration. On comparing leaves with bark, bark was found to be more effective antibacterial agent.

Previous studies have showed that extracts of *Azadirachta indica* were found effective against *Escherichia coli* and *Streptococcus faecalis* with fairly high degree of sensitivity (IZ=18-33 mm) to methanol extracts [29]. *Klebsiella pneumoniae* was found to be intermediate sensitivity. Methanol extract of *Azadirachta indica* showed fairly high degree of sensitivity (IZ=20-33 mm) to all tested bacteria, except *Bacillus subtilis*, which was least susceptible to methanol extract. *E. coli* and *Klebsiella pneumoniae* belongs to Gram-negative bacteria and the most susceptible bacteria to aqueous and ethanol extracts of *Azadirachta indica*. This selective toxicity could be linked to the differences in the composition of the lipid bilayer for the two strains of bacteria. A greater degree of depolarization and hence increased permeability was expressed in the lipid bilayer of the Gram-negative bacteria for this cembranoid, because they contain more lipids in their cell walls [30]. This depolarization effect is suggested to be associated with hydrogen bonding on the hydroxyl group in the carboxylic functionally situated at the C-19 position in the diterpene [31].

The antibacterial activity of *Azadirachta indica* might be due to presence of triterpenoids, phenolic compounds, carotenoids, steroids, valavinoids, ketones and tetra-triterpenoids azadirachtin. Earlier studies on *Azadirachata* claim that a spermicidal fraction of Neem oil (NIM-76) is more effective as an antimicrobial agent as compared to the Neem oil itself especially its effect is less on *Escherichia coli* and *Klebsiella pneumonia* [32]. Antibacterial activity of the extracts of *Azadirachta indica* was effective on *Escherichia coli, Klebsiella pneumoniae* and *Bacillus subtilis* etc. and needs further confirmation [33]. Few authors have reported antimicrobial activity of Neem oil on *Escherichia coli* and *Klebsiella pneumonia* with methanol but not with chloroform and hexane extracts as is influenced by pH of the final extract [34]. A further study is required for isolation and purification of bioactive compounds responsible for the antimicrobial activity.

Antimicrobial activity of Tulsi has been shown in (**Table 3**). At 5 µg concentration, all strains were resistant. At 250 µg, maximum activity was seen in ethanol extract for E. coli followed by *S. aureus*, *B. subtilis* and *K. pneumonia*. Aqueous extract also showed similar activity but to a relatively lower extent. Eugenol (l-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *Ocimum sanctum L.*, has been found to be largely responsible for the antimicrobial property of *Ocimum sanctum L.*[35] In traditional systems of medicine, various parts of *Ocimum sanctum Linn* plant have been used in treatment of bronchitis, bronchial asthma, malaria, chronic fever etc; however a rational approach to this traditional medical practice with modern system of medicine is not much available. In order to establish the therapeutic uses of *Ocimum sanctum L.* in last few decades several Indian scientists and researchers have studied the pharmacological effects of steam distilled, petroleum ether & benzene extracts of various parts of *Ocimum sanctum L.* Essential oils extracted from the leaves of *Ocimum sanctum L.* has been found to inhibit in-vitro growth of *E. coli, B. anthracis* and *P. aeruginosa* showing its antibacterial activity [36]. Therefore, this study confirms these facts.

As all the encountered conditions indicated for these drugs arouse due to various bacterial infections involving different strains of gram positive and gram negative bacteria, it was decided to carry out the study accordingly for two types of extracts. The results show good antibacterial activity at 250 µg concentrations for leaves and bark of Neem as well as leaves of Tulsi.

Table 1: Showing effect of aqueous and ethanol extract of *Azadirachta indica* leaves on various microorganisms

Disc No.	Sample Concentration (µg)	Bacterial Strains (Zone of inhibition in mm)							
		BS B2274		SA 96		EC 739		KP B2405	
		AQ	ET	AQ	ET	AQ	ET	AQ	ET
01	05	08	06	-	06	-	-	-	-
02	25	14	10	09	13	11	14	05	07
03	50	15	13	12	14	14	15	09	09
04	100	17	16	15	16	17	17	11	13
05	250	19	19	18	20	18	20	11	17

AQ – Aqueous Extract; ET – Ethanolic Extract

Table 2: Showing effect of aqueous and ethanol extract of *Azadirachta indica* Bark on various microorganisms

Disc No.	Sample Concentration (µg)	Bacterial Strains (Zone of inhibition in mm)							
		BS. B2274		SA 96		EC 739		KP B2405	
		AQ	ET	AQ	ET	AQ	ET	AQ	ET
01	05	-	-	-	07	-	08	-	05
02	25	8	10	10	14	7	17	6	12
03	50	10	11	13	15	10	19	9	15
04	100	14	15	16	18	12	21	12	17
05	250	16	18	19	20	15	22	12	20

AQ – Aqueous Extract; ET – Ethanolic Extract

Table 3: Showing effect of aqueous and ethanol extract of *Ocimum sanctum* Leaves on various microorganisms:

Disc No.	Sample Concentration (µg)	Bacterial Strains (Zone of inhibition in mm)							
		BS B2274		SA 96		EC739		KP B2405	
		AQ	ET	AQ	ET	AQ	ET	AQ	ET
01	05	-	-	-	-	-	-	-	-
02	25	06	09	06	10	07	09	-	06
03	50	09	12	08	12	10	14	07	09
04	100	11	16	12	18	13	18	10	14
05	250	14	18	12	21	16	22	12	17

AQ -Aqueous Extract; ET- Ethanolic Extract

Table 4: Showing effect Standard Drugs on various microorganisms

Disc No.	Sample Concentration (µg)	Bacterial Strains (Zone of inhibition in mm)			
		SA 96		EC 739	
		CLP	CFN	CLP	CFN
01.	05	12	17	14	20
02.	25	14	19	17	23
03.	50	19	21	23	28
04.	100	20	22	23	28
05.	250	21	22	23	28

CLP – Chloramphenicol ; CFN – Ciprofloxacin

CONCLUSION

Significant antimicrobial activity on *S. aureus, E. coli* followed by *K. pneumonia* and *B. subtilis* was seen in leaves and bark of Neem and Leaves of Tulsi. Although this study investigating the *in vitro* antibacterial activity, the results showed that the ethanol extracts from *Azadirachta indica* leaves and bark as well as *Ocinum sanctum* possessed good antibacterial activity, confirming the great potential of bioactive compounds and are useful for rationalizing the use of this plant in primary health care. *In vivo* data may be helpful in determining the real potential usefulness of this plant for the treatment of infectious diseases.

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