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RESEARCH **P**APER

Antibacterial activity of *Ocimum sanctum* (Tulsi), *Azadirachta indica* (Neem) and *Phyllanthus emblica* (Amla)

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Plant extracts continues the numerous searches for more effective drugs of plant origin which are less toxic and available for low socio-economic population in the treatment of diseases caused by pathogenic bacteria. The potential for developing antibacterial from higher plants appears rewarding as it will result to the development of a phytomedicine to act against microbes. The *Azadirachta indica, Ocimum sanctum* and *Phyllanthus emblica* extracts were tested for antibacterial activity by spread plate method against four pathogens *Escherichia. coli, Staphylococcus aureus, Bacillus subtilis* and *Nessieria flavescenes,*. It was found that gram negative bacteria were largely inhibited by the extract of amlathan that of neem and tulsi. The zone of inhibition was measured which showed that extract of amla was of high antibacterial activity as compared to meem and tulsi. Methanol extracts were more active than the aqueous extract against all the bacteria. The zones of inhibition were ranging from 1- 3.5 cm in diameter. The highest zone of inhibitions (3.5cm) was noted in methanol extract of *P. emblica* against *S. aureus*. The highest yield of methanolic extract was found in *Azadiracta indica* (29.08%). The extract of *Ocimum sanctum* and *Phyllanthus emblica* were most effective against *Escherichia coli* and *Staphylococcus aureus*.

Key words : Antibacterial activity, Plant extract, Zone of inhibition

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INTRODUCTION

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.*, 1999). Plant derived drugs remains important resource especially in developing countries, to combat serious disease. Approximately 62 – 80 per cent of the world's population still relies on traditional medicines for the treatment of common illness. Traditional medicine continues to provide health coverage for over 80 per cent of the world population, especially in the developing world (WHO, 2002). Plants are the major constituents of traditional medicine (Rates, 2001). In fact, plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50 per cent of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982). Natural products play on important role in drug development programmes in the pharmaceutical industry (Baker *et al.*, 1995). *Ocimum sanctum* is active against many infectious human pathogenic bacteria that cause many dangerous diseases such as vomiting, diarrhea, urinary infections, gastroenteritis etc (Eman, 2012). *Azadirachta indica*

shows hypoglycemic effect (Sharma *et al.,* 2011). Neem may help in the search for prevention or cure for AIDS which may possibly be treated by ingesting neem leaf extracts or the whole leaf or by drinking a neem tea (Bhowmick *et al.,* 2010). The extracts of *Phyllanthus emblica* possess several pharmacological properties like anti-viral (HIV, AIDS, HERPES VIRUS, CMV) antimutagenic, anti-allergic, anti-bacterial activities (Khopde *et al.,* 2000). *P. emblica* contains different class of secondary metabolites (Calixto *et al.,*1998). It has been used for anti-inflammatory and antipyretic treatments by rural populations in its growing areas. Malays use a decoction of its leaves to treat fever (Burkill, 1966). In view of the above, the present study was conducted for antibacterial activity of *Ocimum sanctum* (Tulsi), *Azadirachta indica* (Neem) and *Phyllanthus emblica* (Amla).

RESEARCH METHODOLOGY

Collection of plants :

The leaves of *Ocimum sanctum, Azadirachta indica and Phyllanthus emblica* were collected from the School of Forestry, SHIATS, Allahabad.

Sample preparation :

The dried leaves were coarsely powdered and extracted with a mixture of methanol: water (7:3, v/v) by a Soxhlet apparatus at 50°C. The solvent was completely removed and obtained dried crude extract which was used for investigation.

Test pathogens :

Gram negative bacteria, *Escherichia coli* and *N. flevescenes* and gram positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus* were provided by Department of Microbiology and Fermentation Technology, JSBB, SHIATS, Allahabad. All the test bacteria were stored on nutrient agar slaut at 4 ºC. These bacteria were sub cultured for 24 hours before use.

Determination of antimicrobial activity :

The antibacterial activity was determined by the agar well-diffusion method. Overnight grown bacterial culture was transferred to sterile Petri plate with nutrient agar medium and was spread with sterile spreader to create a lawn. Wells of 6mm were punched into the previously seeded NA plates using sterile cork borer. About 100µl of the different extracts were placed in the wells and allowed to diffuse for 2 hrs. at 4ºC and the plates were incubated at 37°C for 24 hrs. The activity was determined by measuring the diameter of the inhibition zones for each well and expressed in millimeter.

RESEARCH FINDINGS AND ANALYSIS

The antibacterial activity of the extracts and their potency was quantitatively assessed by the presence of inhibition zone and zone diameter, respectively. The plants differed significantly in their activity against the tested strains and the best antibacterial activity was observed with three plants namely *Ocimum sanctum, Azadirachta indica* and *Phyllanthus emblica*. The antibacterial activity of many plant extracts has been previously

Table 1: Antibacterial activity of the methanolic extract

Pathogen	Plants and zone of inhibition (cm)		
	O. sanctum	*A. indica*	*P. emblica*
E. coli	2.7	2.8	3.0
B. subtilis	2.2	2.0	2.4
S. aureus	2.4	2.6	3.5
N. flevescenes	1.8	2.1	2.6

Table 2: Antibacterial activity of the aqueous extract

Pathogen	Plants and zone of inhibition (cm)		
	O. sanctum	*A. indica*	*P. emblica*
E. coli	1.9	2.2	1.8
B. subtilis	0	2.0	1.7
S. aureus	1.2	2.1	2.2
N. flavescenes	1.0	1.2	2.0

reviewed and classified as strong, medium or weak (Zaika, 1988).

The present study was designed to obtain preliminary information on the antibacterial activity of three plants of methanol extract as shown in Table 1 and aqueous extract (Table 2). It was found that the methanol extracts of *Ocimum sanctum*, *Azadirachta indica* and *Phyllanthus emblica* showed the maximum inhibition of all the pathogens followed by aqueous extract. The methanol extracts of *P. emblica* showed the higher antibacterial activity as compared with *A. indica* and *O. sanctum* (Table 1). Results indicate that methanol and aqueous extraction is a good method to extract antibacterial compounds found in these species. Solvents (negative control) used for extraction showed no activity against any bacteria tested. The inhibition zones varied depending on the type of extract, plant species and bacterial species. In general, methanol extracts of the three species were found to be more effective than aqueous extracts.

The largest diameter of inhibition zone was observed from methanol extracts of *Ocimum sanctum*, *A. indica* and *P. emblica* against *E. coli*as shown in Fig. 1, *B. subtilis* (Fig. 2), *S. aureus* (Fig. 3) and *N. flavescens* (Fig. 4). The aqueous extracts of *O. sanctum* had less activity against *B. subtilis* and *S. aureus*, whereas methanol extract gave maximum

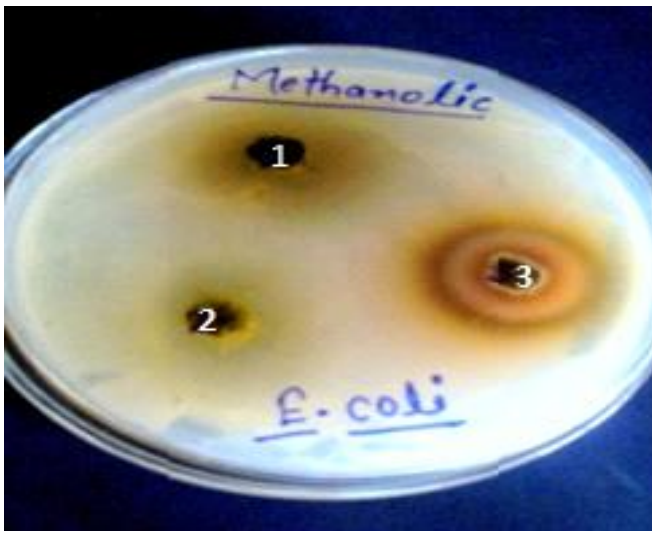


Fig. 1 : ZOI showed in *E. coli*

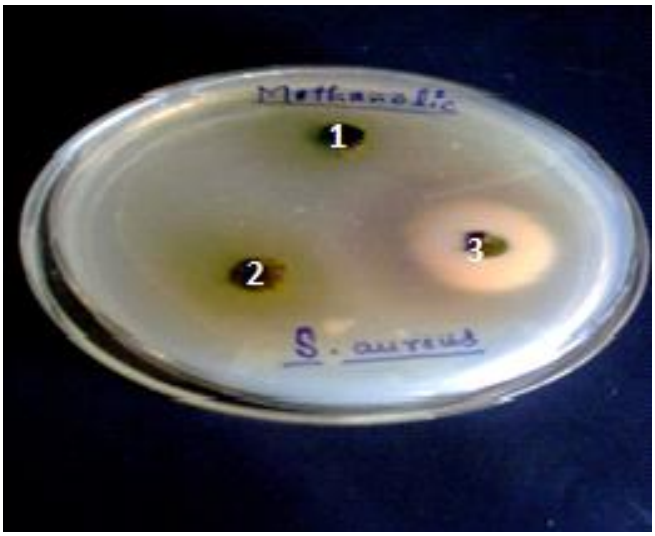


Fig. 3 : ZOI showed in *S. aureus*

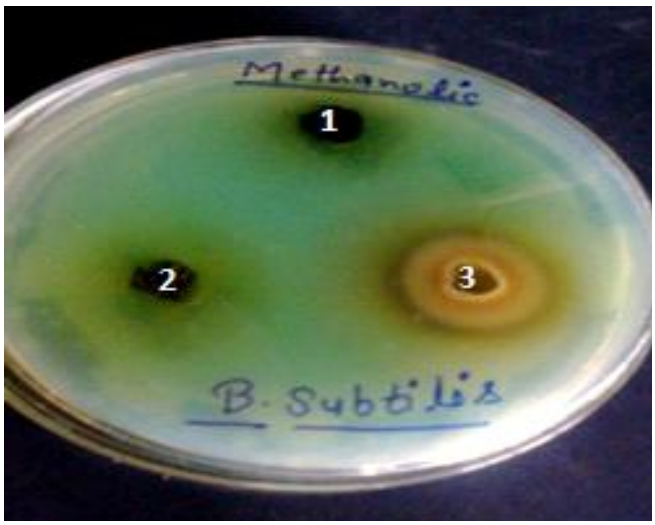


Fig. 2 : ZOI showed in *B. subtilis*

Indications: 1 = *O.sanctum*, 2 = *A. indica*, 3 = *P. emblica*

Fig. 4 : ZOI showed in *N. flavescens*

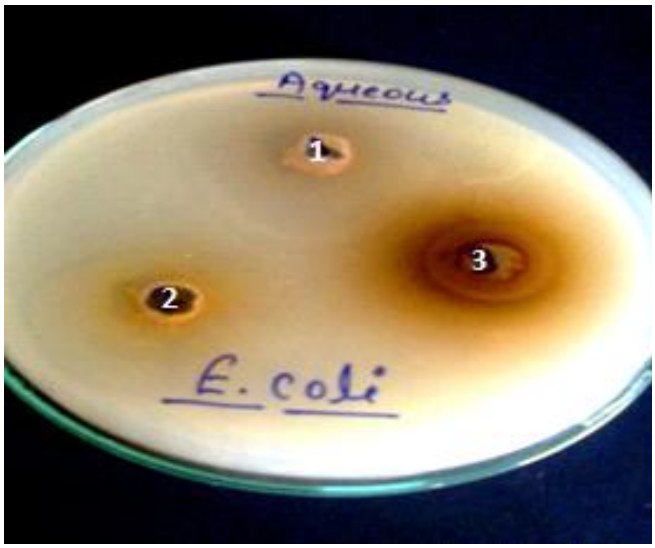


Fig. 5 : ZOI showed in *E. coli*

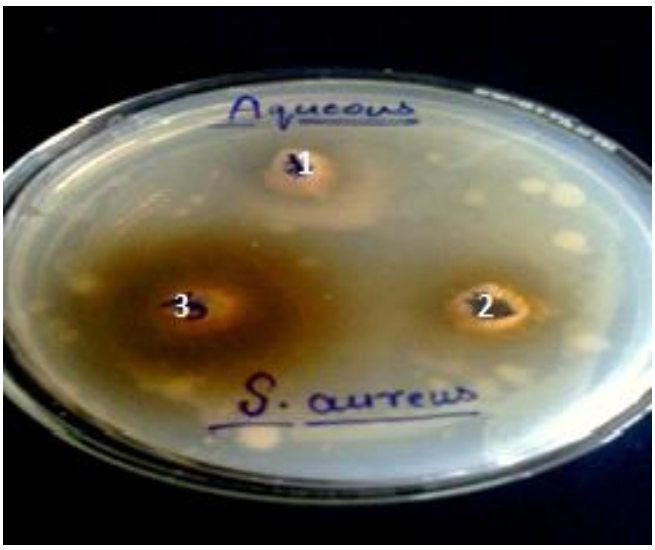


Fig. 7 : ZOI showed in *S. aureus*

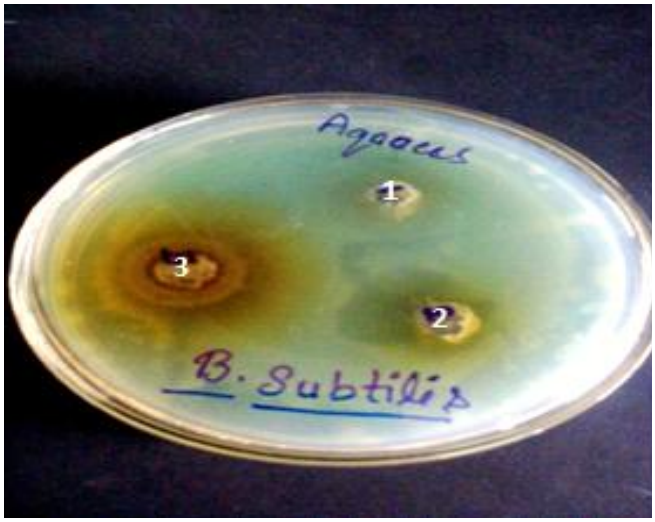
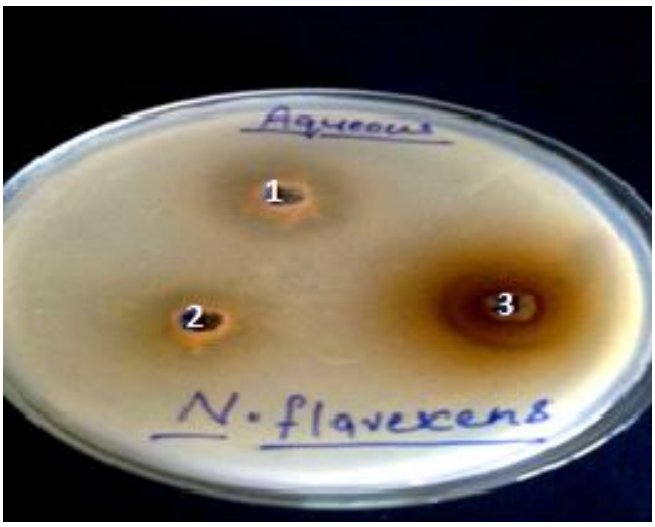


Fig. 6 : ZOI showed in *B. subtilis*



Indications: 1 = *O.sanctum*, 2 = *A. indica*, 3 = *P. emblica*

Fig. 8 : ZOI showed in *N. flavescens*

zone of inhibition in the same pathogen. Vlientinck *et al*. (1995) reported that water extracts of plants do not have much activity against bacteria. However, *O. sanctum* aqueous extracts had no clear zone in against *E. Coli* (Fig. 5), *B. subtilis* (Fig. 6), *S. aureus* (Fig. 7) and *N. flavesens* (Fig. 8) in *O. sanctum* and *A. indica* aqueous solvent. However, the clear zone of water extracts resulted at par with methanol extracts of plant activity against the bacteria tested.

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