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EVALUATION AND COMPARISON OF ANTIBACTERIAL PROPERTIES OF PHYTOCHEMICALS IN SOME COMMON MEDICINAL PLANTS

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ABSTRACT

India is said to be the most biodiversed country. Its Ayurvedic treatments are well known which are even mentioned in the Vedic puranas. In the present study, use of some medicinal plants (*Capsicum frutescens, Neolamarckia cadamba, Ocimum tenuiflorum*, *Psidium guajava L.* and *Azadirachta indica)* have been shown and there antimicrobial property towards some infectious bacteria (*E. coli, S. aureus* and *P. aerugenosa*) in vitro condition. Two controls were also considered (tetracycline and DMSO). In vitro antibacterial analysis was performed by agar-well diffusion method, and the extraction of bioactive compounds was done by solvent extraction method by no. of solvents, keeping if for 24 hours and 48 hours. As a result, the bioactive compound of *Azadirachta indica* plant leaves shows highest antibiotic property among all leaf samples against the different pathogens.

Key words: Antimicrobial, DMSO, *Neolamarckia cadamba*, *Ocimum tenuiflorum*, etc.

INTRODUCTION

Naturally occurring bioactive substances are mainly of plants, animals and mineral origin **[1].** With the increase in the infections in the day to day time, antimicrobial resistance is the major concern for human beings **[2]**. In India there is huge importance of Vedic medicines and home remedies **[3]**. Plants are easily available for use due to its' vast biodiversity. They contain a large variety of bioactive compounds which have lots of antimicrobial, antibacterial, antioxidant and immune boosting properties **[4]**.

Secondary metabolites which are responsible for antimicrobial activity of medicinal plants are soluble in solvent such as ethyl acetate, methanol, ethanol, hot and cold aqueous, etc. **[5]**. Therefore they have been used for extraction of secondary metabolites such as phenolics, flavonoids, tannins, steroids, terpenoides, phlabotannins, etc**. [6].**

Thaipong et. al. (2006) in his journal discussed about the antioxidant property of guava (*Psidium guajava L.*) extracts measured in methanol **[7].** Renaud et al., (1998) and Temple, (2000) discussed that frequent consumption of natural antioxidants in day to day diet are associated with a lower risk of cardiovascular disease and cancer **[8,9]**. Sen et. al. (2016) in their study showed the antibacterial properties of the chilli plant (*Capsicum annuum*) **[10]**. *Neolamarckia cadamba* commonly known as kadam in previous studies found to have antioxidant properties. It also evaluates the membrane stabilizing and anthelmintic properties **[11,12]**. Subramanium et. al., in his study discussed about the importance of Tulsi (*Ocimum tenuiflorum*) commonly known as the Holy Basil and all antimicrobial properties in it **[13].** Neem is proved to be one of the best plants for antibacterial properties **[14,15]**.

The aim of the present studies is to study the bioactive compounds of some commonly used plant species and to know their antimicrobial properties against some pathogenic bacteria.

MATERIALS AND METHODS

Collection of plant materials:

Fresh leaves of all the 5 plants i.e. *Capsicum frutescens, Neolamarckia cadamba, Ocimum tenuiflorum*, *Psidium guajava L.* and *Azadirachta indica* were collected from different regions of Lucknow and were taxonomically recognized by the scientist of MRD Life sciences Lucknow.

They were first washed properly to remove the unwanted dust and were dried in shade to remove all the water molecules present in them. Dried leaves were then blend in the grinder to fine powder **[16, 17].**

Preparation of Plant Extract by Solvent Extraction Method:

Powdered leaves were dissolved in different solvents in 1:10 ratio (1gm powder: 10ml solution) in 10 wash bottles. 5 bottles were then kept for incubation in dark for 24 hours and other 5 bottles for 48 hours. The dissolved powder from solvent was then filtered in the weighed bowls. It was kept at 50-70^{0}C for drying. The bowls with extract were then weighed. The weight of extract was calculated. These extracts were then dissolved in DMSO (twice the weight of extract) and were then transferred in microcentrifuge tubes. Further the extracts were screened for antibacterial properties by using 100mg/ml of each sample **[18]**.

Antibiotic Sensitivity Screening:

NAM (nutrient agar media) plates were prepared. Further the solvents, controls and extracts were screened for their antibacterial properties by using the protocols defined by Caceres et al, 1991 and Singhal et al., 2011 with slightly modification in it. Where, tetracycline and DMSO used as the positive and negative controls. The plates were left for incubation at 37°C **[19, 20].**

RESULTS AND OBSERVATIONS

The metabolites were extracted from the powder of leaves of *Capsicum frutescens, Neolamarckia cadamba, Ocimum tenuiflorum*, *Psidium guajava L.* and *Azadirachta indica* plants and preserved at -20°C for the screening against bacterial pathogens *E. coli, S. aureus* and *P. aeruginosa.* During the screening of solvents against the bacterial strains it was found that they were not able to show inhibitory property. Whereas, a positive control (tetracycline) shows inhibitory activity, mentioned in table 1 and figure 1. Similarly the extracted compounds at 24 hours were not as good as compare to 48 hours extraction process (table 2) (figure 2 and 3).

Table 1: Antibacterial screening of solvents, positive control and negative control.

Samples	Zone of inhibitions (mm)		
	E. coli	*S. aureus*	*P. aeruginosa*
Tetracycline	16.8	18.2	14.3
DMSO	0	0	0
70% ethanol	0	0	0
Benzene	0	0	0
Acetone	0	0	0
Chloroform	0	0	0

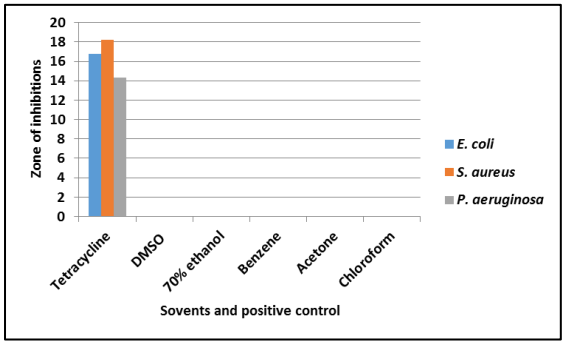
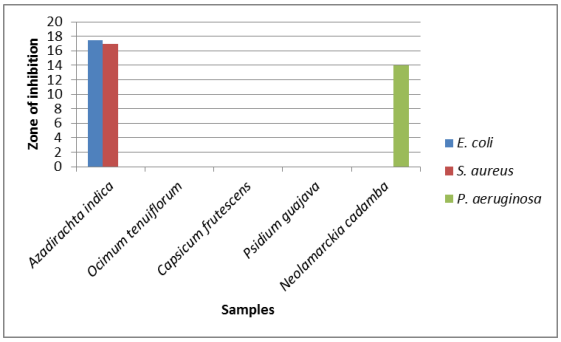


Figure 1: graphical analysis of the antibacterial screening of the solvents and controls against the bacterial strains.

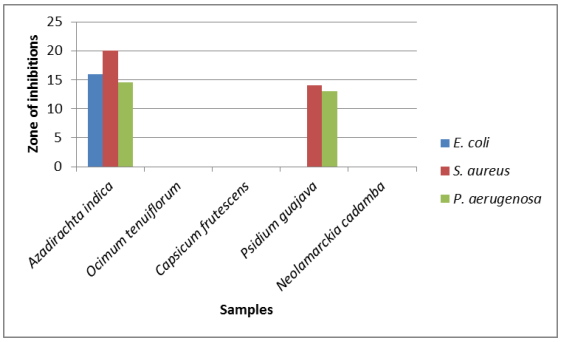
Table 2: Antibacterial screening of extracted metabolites at 24 and 48 hours.

Pathogens	Zone Of inhibition (mm)				
	A	B	C	D	E
24 hours extraction process					
Ec	17.5	0	0	0	0
Sa	17	0	0	0	0
Pa	0	0	0	0	14
48 hours extraction process					
Ec	16	0	0	0	0
Sa	20	0	0	14	0
Pa	14.5	0	0	13	0

Where Ec: *E. coli,* Sa*: S. aureus,* Pa*: P. aeruginosa, A: Azadirachta indica, B: Ocimum tenuiflorum, C: Capsicum frutescens, D: Psidium guajava, E: Neolamarckia cadamba*

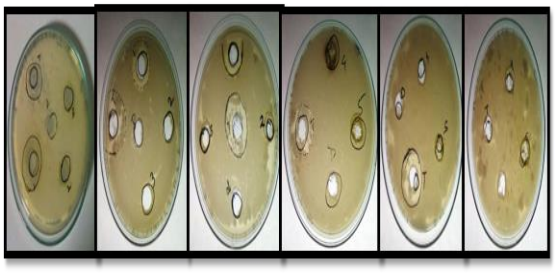


a: antibacterial analysis of 24 hours extracted compounds

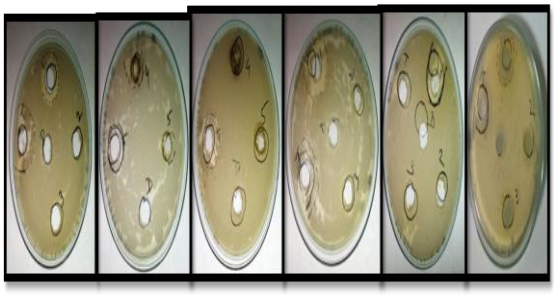


B: antibacterial analysis of 48 hours extracted compounds

Figure 2: graphical representation of the screening of 24 and 48 hours extracted metabolites.



a: screening of 24 hours extracted compounds as compared with the solvents and controls



b: screening of 48 hours extracted compounds as compared with the solvents and controls

Figure 3: clear zones indicate the inhibitory activity of the extracts, controls against the bacterial strains.

DISCUSSION

Five plants were studied for antimicrobial activity against three Gram-negative and two Gram-positive bacteria, using different solvents for extraction process. The antibacterial properties of extracted metabolites were carried out at different time intervals, 24 hours and 48 hours.

During the analysis of the extracts of 24 hours, there was a positive inhibition were expressed by the *Azadirachta indica* extract, 17.5 mm, 17 mm against *E. coli* and *S. aureus.* Similarly *Neolamarckia cadamba* shows expressive results against *P. aeruginosa* by showing 14 mm of clear inhibitory zone as labelled in table 2. DMSO taken as a negative control showed no interference in pathogens growth, similarly no interference with the working of bioactive compounds.

Whereas in 48 hours of extraction (Table-2), bioactive compounds of *Azadirachta indica* showed inhibitory activity against all the three pathogens, *E. coli* (16 mm), *S. aureus* (20 mm), and *P. aerugenosa* (14.5), same with bioactive compound of *Psidium guajava* showed property against *S. aureus* (14 mm) and *P. aeruginosa* (13 mm), rest does not show any antibacterial activity. It should be taken into consideration that the extract of *Neolamarckia cadamba* worked in 24 hours of extraction and lost its properties in 48 hours of extraction, whereas extract of *Psidium guajava* worked after 48 hours of extraction.

Azadirachta indica extract, after 48 hours of extraction showed better results and worked against *P. aerugenosa.*

Subramanium et. al., in his study on *Ocimum tenuiflorum* showed the property over the same pathogen by different solvents but in the present study no effective results was observed when the extraction solvent was benzene. Hence, benzene can be considered a non-working solvent in the *Ocimum tenuiflorum* extraction. Phytochemicals contains several metabolites like tannins, flavonoids, phenols and other aromatic and aliphatic compounds, which in some or the other way helps us to fight pathogens. They play a vital role in our life, only the thing required is to use them properly.

Victor, et. al. (2012) conducted a similar study on *Azadirachta indica* plant and found that it is not resistant to *Pseudomonas* in 3 solvents, different from this study. Hence, the *Azadirachta indica* is found working more effectively in 70% ethanol, showing resistance to *Pseudomonas*. Various other studies have also been conducted to recognise such use of different types of plants that are either medicinal or non-medicinal.

CONCLUSION

The study revealed about the various bioactive compounds and its effect on different pathogens in different catastrophic scenarios. This also reveals about the importance of solvent during solvent extraction of bioactive compounds.

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