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EVALUATION OF ANTIBACTERIAL ACTIVITY OF BAUHINIA VARIEGATE LEAF EXTRACT USING DISK DIFFUSION METHOD AGAINST SOME HUMAN PATHOGENIC BACTERIA'S

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ABSTRACT

Microbial resistance to antibiotics is highly increasing during the three last decades. Evaluation of natural products to access new and effective antimicrobial agents is one of the scientific strategies to combat drug-resistant pathogens. With this perspective, leaf extracts of Bauhinia vareigata which had documented uses in traditional medicine, Bauhinia vareigata leaf extract tested against some Gram-negative and Gram-positive bacteria such as Bacillus subtilis, Bacillus cereus, Bacillus frimicutes, Escherichia coli, Entrobacter, Klebsiella, Escherichia coli. The antimicrobial activities were evaluated using agar disc diffusion method. The results indicated that the antimicrobial of Bauhinia vareigata Linn inhibit the growth of microorganism's as dose dependently manner. They appear results confirm that the antibacterial activity of Bauhinia vareigata leaf extract in present test system. It is concluded that this plant can be indispensible source for secondary metabolites.

KEYWORDS: Bauhinia vareigata, methanolic, Pathogenic Bacteria, E. Coli, Leaf, antibiotics.

1. INTRODUCTION

The relatively large Bauhinia genus (Family: Caesalpiniaceae) consisting of trees, climbers and shrubs is distributed in a wide range of geographic locations. Certain Bauhinia species have a long history of traditional medicinal applications (Valdir, 2009). The plant Bauhinia

variegate Linn. (Caesalpiniaceae) commonly known as Mountain Ebony is a medium-sized, deciduous tree, found throughout India. It is widely used in folklore medicine. Its bark, root, leaves, seeds and flowers are used for their medicinal properties. It has been used in dyspepsia, bronchitis, leprosy, ulcer, to prevent obesity, as an astringent, tonic and anthelmintic (The Wealth of India, 1959).

There are a few reports on the use of plants in traditional healing by either tribal people or indigenous community (Sandhy et al., 2006; Ayyanar and Ignacimuthu, 2005; Rajan et al., 2002; Natarajan et al., 1999 and Ignacimuthu et al., 1998). The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem (Austin et al., 1999). Natural products of higher plants may give a new source of antimicrobial agents.

There are many research groups that are now engaged in medicinal plants research (Samy et al., 1998; Hamil et al., 2003; Motsei et al., 2003). Silver and Bostian (1993) have documented the use of natural products as new antibacterial drugs. There is an urgent need to identify novel substances active towards highly resistant pathogens (Recio, 1989; Cragg et al., 1997). In an effort to discover new compounds, many research groups screen plant extracts to detect secondary metabolites with the relevant biological activities. In this regard, several simple bioassays have been developed for screening purposes (Hostettmann, 1991). The present study was carried out on the phytochemical and antibacterial activity of leaf and bark of B. variegata. The current study investigates the anti-microbial potential of *B. variegata* leaf extract against some gram positive and gram negative bacterias.

2. MATERIALS AND METHODS

Plant materials: The *B. variegata* leaf were collected from local garden of Raipur, Chhattisgarh and dried for few days in shade, which were then powdered and preserved in airtight bottles for further studies.

Extract preparation: *B. variegata* leaf (20g) was extracted in 50% of methanol and Millipore water solvent the supernatant was collected and concentrated in water bath at 40-50 C. The dried powder was kept in air tied box.

Microorganisms: The tested microorganisms included the Gram positive bacteria; *Bacillus subtilis, Bacillus cereus, Bacillus frimicutes* and Gram negative bacteria; *Escherichia coli, Entrobacter, Klebsiella, Escherichia coli*. These bacteria's strains were procured from National Chemical Laboratory (NCL), Pune, India. The bacteria were grown in the nutrient broth at 37º C and maintained on nutrient agar slants at 4º C.

Antibacterial assay: Antibacterial activity of *B. variegata* leaf extract was determined by agar disk diffusion method (Nair, et, al., 2005) at four concentrations i.e., 100, 75, 50 and 25 mg/ml. Muller Hinton agar was prepared according to the manufacturer's instructions and the plates were seeded with appropriate microorganisms (Gram positive bacteria; *Bacillus subtilis, Bacillus cereus, Bacillus frimicutes* and Gram negative bacteria; *Escherichia coli, Entrobacter, Klebsiella, Escherichia coli*). Discs of 6 mm diameter were prepared from Whatmann filter paper No. 24 and sterilized. The discs were then impregnated with the extracts and solvent DMSO. Antibiotics for Gram positive (NX – Norfloxacin, OF-Ofloxacin, E-Erythromycin, CFM- Cefixime) and Gram Negative (NX–Norfloxacin, OF-Ofloxacin, E-Erythromycin, CFM- Cefixime).Bacteria were used as standard. The plates were incubate at 37º C for 24 hrs and the zones of inhibition were measured with a measuring scale. Above experiment was carried out in triplicate for their confirmation.

RESULT

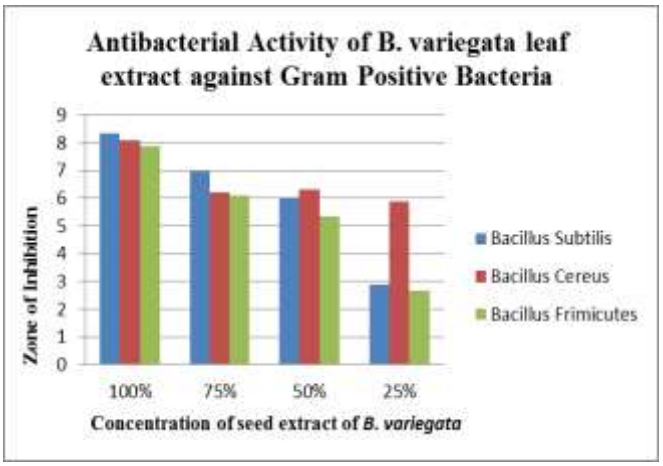
The result of microbial growth was considered as zero hour and further accordingly reading was taken. Our present study show that antimicrobial activity of 50% methanolic extract of *B. variegata* leaf extract against B. subtilis is best in 100% concentration after 12 hrs. 9.12 mm zone of inhibition. Although 75% concentration is having mild effect as 6.78 mm zone of inhibition. In B. cereus is best in 100% concentration of extract is 8.40 mm and in 50% concentration is 6.33 mm zone of inhibition. In B. frimicute the 100% concentration is having 7.88 mm zone of inhibition. In E. coli 100% concentration show maximum activity of 7.00 mm and in 75% also show a good zone of inhibition 6.77 mm. In Klebsiella 100% concentration show minimum 5.33 zone of inhibition. In Entrobacter 100% and 75% show activity of 8.22 mm zone of inhibition.

The above observation suggested that the different concentration (50%, 75%, 100%) were having good anti-bacterial activity against some gram positive (+) bacteria B. subtilis, B. cereus, B. frimicute and some gram negative bacteria E. Coli, Klebsiella, Entrobacter. The selected plant extract is showing great activity against all microorganisms. On comparing the

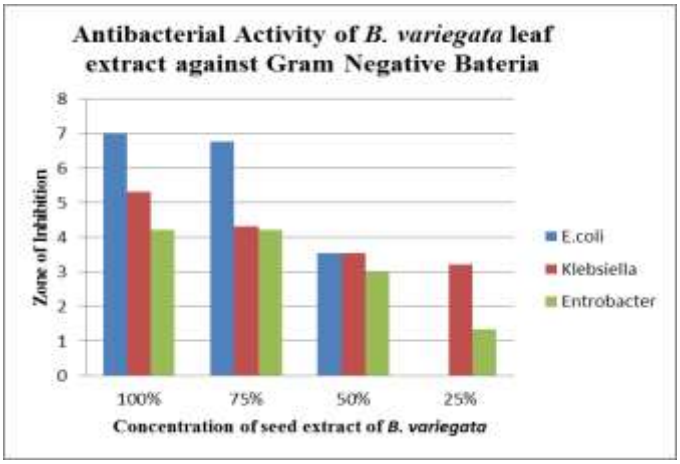
zone of inhibition of extract to that standard antibiotic extract showed better activity than Ciprofloxacin (CIP), Doripenem (DOR), Ofloxacin (OF), Maxifloxacin (OM) in these conditions.

Table 1: The study of anti-bacterial activities of *Myristica fragrans* extracts using disk diffusion method (Mean ± SE).

SI	Bacterial Stain	Bacteria use	Zone of inhibition (In MM)			
			100%	75%	50%	25%
1.	Gram Negative (-)	*Bacillus subtilis*	9.12±0.56	6.78±0.33	5.99±0.77	2.88±1.49
		Bacillus cereus	8.40±0.95	6.21±0.29	6.78±0.84	5.88±0.22
		Bacillus frimicute	7.88±0.77	6.10±0.10	5.33±0.69	2.66±0.66
2.	Gram positive (+)	*E. coli*	7.00±0.57	6.77±0.69	6.55±0.38	2.36±0.87
		Klebsiella	5.33±1.83	5.33±1.07	3.55±1.22	3.21±1.23
		Enterobacter	4.22±0.58	8.22±0.10	2.99±1.50	1.33±1.33



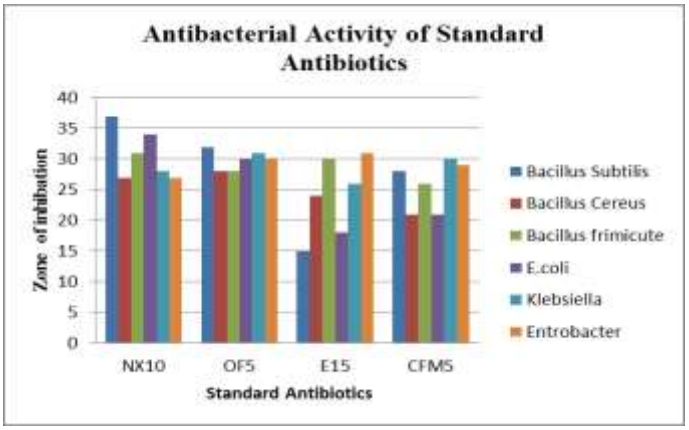
Graph 2: Antibacterial activity of myristica fragrans seed extract against gram positive bacteria.



Graph 3: Antibacterial activity of *b. variegata leaf* extract against gram negative bacteria.

Table 2: The study of anti-bacterial activities of standard antibiotics using disk diffusion method.

SI	Bacterial stain	Bacteria use	Zone of inhibition (In MM)			
			NX10	OF5	E15	CFM5
1.	Gram positive (+)	*Bacillus Subtilis*	37.00	32.00	15.00	09.00
		Bacillus cereus	27.00	28.00	24.00	21.00
		Bacillus frimicute	31.00	28.00	30.00	26.00
2.	Gram Negative(-)	*E. coli*	34.00	30.00	18.00	21.00
		Klebsiella	28.00	31.00	26.00	29.00
		Enterobacter	27.00	30.00	31.00	29.00



Graph 1: Showing the antibacterial activities against standard antibiotic.

DISCUSSION

Their mode of antimicrobial action is related to their ability to inactivate microbial adhesion, enzymes and cell envelope proteins. It is known that medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. Over the past few years, medicinal plants have regained a wide recognition due to an escalating faith in herbal medicine. In view of its lesser side effects compared to allopathic medicine in addition, the necessity of meeting the requirements of medicine for an increasing human population (Akrayi, 2012).

Indian systems of medicine such as Ayurveda and Siddha uses majority of the crude drugs that are of plant origin. It is necessary that standards have to be laid down to control and check the identity of the plant an ascertain its quality before use. A detailed pharmacognostic evaluation therefore is highly essential prerequisite (Ramana, 2007).

Our present result of microbial growth was considered as zero hour and further accordingly reading was taken. Our present study show that antimicrobial activity of 50% methanolic extract of *B. variegata* leaf extract against B. subtilis is best in 100% concentration after 12

hrs. 9.12 mm zone of inhibition. Although 75% concentration is having mild effect as 6.78 mm zone of inhibition. In B. cereus is best in 100% concentration of extract is 8.40 mm and in 50% concentration is 6.33 mm zone of inhibition. In B. frimicute the 100% concentration is having 7.88 mm zone of inhibition. In E. coli 100% concentration show maximum activity of 7.00 mm and in 75% also show a good zone of inhibition 6.77 mm. In Klebsiella 100% concentration show minimum 5.33 zone of inhibition. In Entrobacter 100% and 75% show activity of 8.22 mm zone of inhibition.

The above observation suggested that the different concentration (50%, 75%, 100%) were having good anti-bacterial activity against some gram positive (+) bacteria B. subtilis, B. cereus, B. frimicute and some gram negative bacteria (−) E. Coli, Klebsiella, Entrobacter. On comparing the zone of inhibition of extract to that of standard antibiotic extract showed better activity than Ciprofloxacin (CIP), Doripenem (DOR), Ofloxacin (OF), Maxifloxacin (OM), in this condition.

The combined effect of tannins, glycosides and flavonoids may be responsible for the antimicrobial effect of the extract seen in this study. The result of present investigation clearly indicates that the antibacterial activity with the species of the plants and plant material used. The study most useful for ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs.

CONCLUSION

Plants have been used as healers and health rejuvenators since time immemorial. Even now, WHO recognizes that medicinal plants play important role in the health care of about 80% World population in developing countries and depend largely on traditional medicines, of which herbal medicine constitutes the most prominent part.

In our experiment the microbial growth was considered as zero hour and further accordingly a reading was taken. The information collected above on the use of *B. variegata* across the world having similarity with available literature. In the last few year, ethanol - botanical and traditional applications of natural compounds, especially of herbal origin received most of attention as they are well tested for their efficacy and generally believed to be safe and effective for human.

Experiment for antibacterial study was also done which show a better antibacterial activity against all the six test gram-positive and gram-negative bacteria species used and show antibacterial susceptibility to *B. variegata* leaf extract with clear zone of inhibition. So, in future it can be used as alternate to synthetic antibiotics. Much effort has needed to increase *B. variegata* as dietary supplement in food so as to acquire harm generated by free radicals and resist the human pathogenic bacterial disease.

Activity of the spices against pathogens that appear to be resistant to many antibiotics shows that spices have a glowing future in the treatment of illnesses caused by the food pathogen investigated in this study. However it is necessary to determine the toxicity of the active constituents, their side effect and pharmaco-kinetic properties.

Amongst the plant species investigated, methanol extract of Bauhinia variegata bark showed the most remarkable activity. The polarity of the solvent seems to play an important role in exhibiting potential antibacterial activity. Here, alcohol extracts of *Bauhinia variegata* L. showed remarkable activity against some medically important bacterial strains. In addition such results justify the traditional use of *Bauhinia variegata* L. Further phytochemical studies for identification and elucidation of active constituent in plant material tested in expected to serve as lead in the development of novel bioactive antimicrobial compound.

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