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Study of Phytochemical study and Antibacterial activity of *Bahunia verigata leaf* extract against Some human pathogenic Bacteria

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ABSTRACT

The medicinal plants have been one of the major sources of medicines since the beginning of civilization. There is high demand for plant based medicines, nutraceuticals and cosmeceuticals all across the globe. Herbal medicines are becoming more and more popular in recent years with their over increasing acceptability in both developing and developed countries. This study conducted to investigate the antimicrobial and phytochemical properties extracts of ethanolic extract of Bauhinia variegata Linn. Leaf extract to justify the traditional claim endowed upon this herbal drug as a rasayana in Ayurveda. This study conducted to evaluate the phytochemical study of Bauhinia variegata. The antibacterial activity of Bauhinia variegate leaf extract was also determine using agar disk diffusion methods at different concentration using gram positive and gram negative bacteria. The leaves of leaves of Bauhinia variegata Linn showed the presence of Alkaloids, Saponin, Terpenoids, Flavonoids, Protein, Glycoside, Phytosterol and Tannin. A significant correlation was also observed between zone of inhibition and concentration of extract. These results confirm the antibacterial activity of Bauhinia variegata and support the traditional use of the plant in therapy of bacterial infection. The results of the present study indicated that Bauhinia variegata has potential antibacterial compounds which can be further characterized for identifying new lead molecules against various pathogenic microorganisms. Emergence of drug resistance and reduced efficiency of most of the antibacterial drugs necessitates identification of potential lead molecules with new molecular targets and mechanism of action.

Key words: *Phytochemical, Antibacterial activity, Disk Diffusion method, Gram Positive and Negative, Bauhinia variegata.*

INTRODUCTION

Plants are potent biochemists and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc (Gordon and David, 2001) i.e. any part of the plant may contain active components. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct (Wink, 1999). The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. In particular, the search for components with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by

antibiotic-resistant microorganisms (Davies, 1994). Hence, there is a constant need for new and effective therapeutic agents. Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs. Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found. Initial screening of plants for possible antimicrobial activities typically begins by using crude aqueous or alcohol extraction and can be followed by various organic extraction methods. Since nearly all of the identified components from plants are active against microorganism are aromatic or saturated organic compounds, they are often obtained through initial ethanol or methanol extraction (Vilegs et al., 1997). The research on the medicinal plants should be extended with the identification of the active principles in the plants. Scientific examination of the remedies could lead to standardization and quality control of the products to ensure their safety. It is after such evaluations that they can be approved for use in the primary health care. Such research activities could also lead to the development of new drugs as in the past.

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).

Bauhinia variegata L. (Caesalpiniaceae) having Kachnar as the local Indian name, was evaluated for the preliminary antibacterial activity and phytochemical analysis. The different extracts of Bauhinia variegata L. were screened for potential antibacterial activity against some medically important bacterial strains.

MATERIALS AND METHODS

Plant Materials: The Piper betel leaf were collected from local market of Raipur, Chhattisgarh and dried for few days in shade, which were then powdered and preserved in airtight bottles for further studies.

Extract Preparation: Piper betel 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.

Phytochemical screening of the extract:

Phytochemical screening was performed to test for alkaloids, saponins, glycoside, proteins, Phytosterols, flavonoids terpenoids, tannins fixed oil and fats.

Test for Alkaloids: A small portion of the extract was stirred separately with 1 ml of dilute Hydrochloric acid and filtered. The filtrate was treated with Dragandroff's reagent. Appearance of organic precipitate shows the presence of alkaloids.

Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Glycosides: Small quantity of the extract o was hydrolyzed with 5ml Hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Fehling's test. To 2ml of Fehling's solution (1ml of Fehling's A and 1 ml of Fehling's B solution), 2ml of extract was added, mixed well and boiled. Appearance of yellow or red color precipitate indicates the presence of reducing sugars.

Test for Proteins: Small quantity of the extract was dissolved in 5 ml of water and subjected to Xantho protein test. To 3 ml of the extract, 1ml of concentrate Nitric acid was added. A white precipitate was obtained. The solution was heated for 1minute and cooled under tap water. It was made alkaline by excess of 40% NaOH. Appearance of orange precipitate indicates the presence of protein.

Test for Phytosterol: Salkowski test was done for the detection of phytosterols. In this test, 1 ml of concentrated Sulphuric acid was added to the 1g plant extract and allowed to stand for 5 minutes. After shaking, formation of golden yellow color in the lower layer indicates the presence of phytosterols.

Test for Flavonoids: The extract was treated with concentrated Sulphuric acid. Appearance of yellowish orange show the presence of anthocyanins, yellow to orange color show the presence of flavones, and orange to crimson show the presence of flavonons.

Test for terpenoids (Salkowski test): 5 g of each extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for tannins: About 0.5 g of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

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 Piper betel 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 results of phytochemicals tests carried out for *Bauhinia variegata* with different solvents are present in Table 1. The present study exhibited the presence and absence of phytochemical compounds in each solvent extract. It was found that methanol showed maximum number of phytochemicals when compared to Chloroform, Petroleum ether and Acetone.

Table 1: Show the Phytochemical study of *Bauhinia variegata* extract

Presence = +, Absent= -

Sl	Components	*Bauhinia variegate Extract*			
		Methanol	**Petroleum Ether**	**Acetone**	**Chloroform**
1	Alkaloids	+	+	+	+
2	Saponin	+	+	+	+
3	Terpenoids	+	+	+	+
4	Flavonoids	+	+	+	+
5	Protein	+	-	+	+
6	Glycoside	+	+	-	+
7	Phytosterol	+	-	-	-
8	Tannin	+	-	-	+
9	oils and fats	+	-	+	-

The initiation of microbial growth was considered as zero hour and further accordingly reading was taken. Our present study show that antimicrobial activity of 50% metabolic extracts of *Bauhinia variegata* leaf against *Enterobacter* is best in 100% concentration after 12 hrs. 13.66 mm zone of inhibition. Although 75% concentration is having mild effect as 09.43 mm zone of inhibition. In *Bacillus subtilis* and *Bacillus cereus* is best in 100% concentration of extract is 10.25 & 12.66 mm and in 75% concentration are 14.23 and 11.22 mm zone of inhibition respectively. In *E. coli* 100% concentration show maximum activity of

14.10 mm and in 75% also show a good zone of inhibition 13.23 mm. In Klebsiella 100% concentration show minimum 12.16 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*. Thus the extract is showing varying 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 2: showing the study of antibacterial activity of Bauhinia variegata leaf extract 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***	10.25±0.87	14.23±1.82	11.33±2.32	08.00±0.20
		Bacillus cereus	12.66±0.65	11.00±0.10	14.66±2.40	06.00±1.15
		Bacillus frimicute	11.00±1.15	09.00±1.73	14.66±2.60	05.66±2.40
2.	**Gram positive (+)**	***E. coli***	14.10±0.87	13.23±0.46	07.0±0.00	06.23±1.32
		Klebsiella	12.16±0.87	08.00±0.98	7.66±0.65	06.73±0.32
		Enterobacter	13.66±1.32	09.43±0.847	7.66±1.19	07.73±1.90

Higher and aromatics plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts [1]. Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases [2].

DISCUSSION

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 variegata* 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). Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. *Bauhinia variegata* an indigenous medicinal plant, has a folk (Siddha and Ayurveda) reputation in the rural areas of southern India and is a member of family Piperaceae.

The antimicrobial activity of 50% methanolic extracts of *Bauhinia variegata* leaf against *Enterobacter* is best in 100% concentration after 12 hrs. 13.66 mm zone of inhibition. Although 75% concentration is having mild effect as 09.43 mm zone of inhibition. In *Bacillus subtilis* and *Bacillus cereus* is best in 100% concentration of extract is 10.25 & 12.66

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Another set of experiment, the plant extract were screened for the presence of major secondary metabolite classes such as Alkaloids, Flavonoids Saponin, Terpenoids, Tannin, Glycosides, Phytosterol, and proteins according to common Phytochemical methods. The test was based on visual observation of the change in color or formation of precipitate after the addition of specific reagent.

Amongst the plant species investigated, methanol extract of Bauhinia variegata leaf 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.

SUMMARY

The exploration of new medicinal properties of various plant species has induced the attention of the scientists towards the biologically active compounds since the last couple of decades. The reason behind this is that the bioactive compounds possess potent pharmacological activities and have low or no toxicity.

Our reports also showed that the leaf of this plant contains many beneficial bioactivities and its extract has a great potential as antimicrobial agent. In this study to determine the antibacterial activity of *Bauhinia variegata* at different dose level form with an in vitro study model which may be helpful in developing new novel drugs. Another set of experiment, the plant extract were screened for the presence of major secondary metabolite classes such as Alkaloids, Flavonoids Saponin, Terpenoids, Tannin, Glycosides, Phytosterol, and proteins according to common Phytochemical methods. The test was based on visual observation of the change in color or formation of precipitate after the addition of specific reagent.

In conclusion from the recorded data, it is demonstrated that the methanolic extract of leaves of *Bauhinia variegata* has promising antibacterial effect. The extract also contains very prominent amount of phenolic compounds which may be responsible for its potent antibacterial activity. As the current study confirmed that leaves of *Bauhinia variegata* showed several biological activities, so taking into consideration of all the findings it can be mentioned that *Bauhinia variegata* leaves can contribute major role in drug research. The plant may be further explored for its phytochemical profile to recognize the active constituents accountable for its versatile activities.

The overall results of the antibacterial activity of various extracts of *Bauhinia variegata L.* at three concentrations studied reveal that deffated extracts showed better activity than those without deffating. The polarity of the solvent seems to play an important role in exhibiting potential antibacterial activity. Here, acetone and methanol 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 it also supports some of the phytochemical and pharmacological investigation of this plant carried by many researchers. The results suggest that traditional folk medicine could be used as a guide in our continuing search for new natural products with potential medicinal properties.

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