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PHYTOCHEMICAL ANALYSIS ANDANTIBACTERIAL POTENTIAL OF LEAF EXTRACT OF *BAUHINIA* LINN.: AN ETHNOMEDICINAL PLANT

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ABSTRACT

Various agents in medicinal plants are used in the same way as antibiotics in treatment of acquiring resistance in bacteria.The main objective of this study is to check out the bactericidal action of leaves of *Bauhinia variegate*Linnagainst medically important one Gram positive (*Staphylococcus aureus)* and three gramnegative bacterial strains *(Proteus vulgaris, S. typhi* and *E. coli)* by the method of disc diffusion. This method showed 24mm maximum zone of inhibition against *Staphylococcus aureus*, 18.5mm against *Proteus vulgaris*, 20mm in *Escherichia coli* and 17.5 mm in *Salmonella typhi*.Zone of inhibition of extract was compared with standard antibiotic Cephalosporin.The phytochemical screening of ethanolic extract revealed many secondary metabolites existence,like flavonoids, terpenoids, tannins, steroids, glycosides and anthraquinones. Various plants are considered medicinal importance as having microbial activity due to containing many secondary metabolites. So, the development of research achievements in pharmacy is enhanced due to naturally occurring bioactive compounds of these plants.

1. INTRODUCTION

Antibacteria Theconsiderable originsfor the invention of new and competent drugs that utilizearound the world are traditional curative systems of herbal therapy[1]. The rate of acquiring drug resistance is rising dangerously in last decades is one of the possible reason of health problems. Drugs resistance have been caused some antibiotics to become almost obsolete and consequently new drugs must be desired [2, 3]. Though a bulk of new antibiotics have been created bypharmacological industries in the previous three decades, yet microorganisms have developed resistance against these drugs. In general, the genetic capability of bacteria to spread and achieve resistance to drugs, may be appropriate as therapeutic agents [4]. Compounds within the herbal plants have been analyzed by numerous studies are effective antibiotics [5].The considerable and challengingmission for medicinal chemistswill consequently the exploring for new antimicrobial agents.

The important resources of natural antimicrobial compounds have considered as a large number of medicinal plants [6]. A vast untappedfountain for medicines areantimicrobials and antibacterial (plant based)and hence have colossal therapeutic potential. Therefore, curiosity on antimicrobial activityexhibitingin higher plant extracts has increased recent years [7-9]. Synthetic drugs are associated with many side effects, while antimicrobials drugs that found in plants have ability to reduce these adverse effects as well as improve treatment of these infectious disorders [10]. In pathogenic organisms, increased expression of multi drug resistance is due to indiscriminate use of antibiotics [11]. The synthetic antibiotics not only have high cost, but also have adverse side effectssuch as, allergic reactions,hypersensitivity immunosuppression etc, and in treating these infectious diseases are major burning global issues [12]. Although the commercial antibiotics had been invented by pharmacological industries time to time in considerable large number but pathogenic microorganism acquired resistance against these drugs at high rate and ultimately lead toward the situation of multi drug resistant in microorganism [13]. Diarrhea and septicemias are caused by Escherichia coliand it can also infect the gall bladder, surgical wounds, meninges, skin lesions, and the lungs especially in debilitate and immunodeficient patients. Urinary tract infections andwound infections are caused by*Proteus spp.*in both the old and young men by following cystoscopy, and in ulcers pressure sores type disorders they are secondary invader [14]. The hospital acquired infections are majorly caused by one of the class of gram positive round shaped bacteria that are *Streptococcus aureus* [15]. The surgical site infection and lower respiratory tract infections are primarily caused by it, pneumonia and myocardial infarctions are also caused by it [16]. The emerging resistant against antimicrobial drugs of especially Penicillin (β lactams) in *S.aureus* resulted in difficult to treat its infections [15]. *Salmonella* species is the source of salmonellosis disease [17]. The symptoms likeacute onset of fever,diarrhea, abdominal pain, nausea and sometimes vomiting are the clinical sources of human salmonellosis.Salmonellosis disease is most commonly associated in animals especially in swine and poultry [18].

In national health care programmers traditional/herbalremedies are majorityrecommends, boosts, and promotes by World Health Organization (WHO) because these drugs aresafe, easily available at low cost, and people have faith in them [19]. *Bauhinia variegata* Linn (Leguminosae) has been selected in order to augment the range of naturallyoccurringantibacterial agents. *Bauhinia variegata*, is the scientific name of Kachnar, is medium and of short duration tree found in India, China,Pakistan, and Nepal [20].For their medicinal properties, its bark, roots, seed, flowers are mainly used. The cooling, acrid, constipating,anthelmintic, depurative, and anti-inflammatory are the medicinal properties of the roots and barks [21]. They are useful in treating certain diseases such as diarrhea,skin disease, dysentery, leprosy,wound, intestinal worms, ulcers,cough, tumors and antidiabetic [22,23]. In folklore medicine, various diseases like inflammation is easily managed by the use of this plant [24]. In present study, *Proteus vulgaris*, *Escherichia coli*, *Salmonellatyphi* and *Streptococcus aureus* are taken as tested strains to check the antibacterial activity of plant extract *Bauhinia variegata*.

2. MATERIAL AND METHOD

2.1. Plant collection and authentication

Sample leaves were collected in month of June from Emerson College Bosan Road, Multan. The shape of different parts of plant (i.e) apex, margin, leaves base etc was examined morphologically etcby taxonomic experts from Department of Botany, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan.

2.2. Plant extract preparation

Leaves were washed with tap water and placed under shaded area for drying. These were collected as they became crispy and subjected to grind to obtain a fine powder. This was, then, stored in polythene bag to use in further processes. For extraction purpose 300gm of powdered leaves socked in ethanol-distill water (70-30%, v/v). Sample solution was tightly closed with lid to avoid evaporation of ethanol and well shook to homogenize all mixture by placing it at room temperature. During this mixture was occasionally shaken by hand. After 7-15 days socking process vegetative debris was passed through Whattman filter paper. To get crude extract solvent was evaporated with rotary evaporator under reduced pressure. Hence a thick sticky concentrated paste was collected in bottle jar which was kept at 4°C until tested or utilized.

2.3. Antimicrobial assay

Bactericidal action of extracts of plants was examined by using disc diffusion method [25,26]. Briefly, nutrient agar and MacCONKEY agar are used media to culture the bacteria. Sterilized cotton swab dipped into freshly prepared inoculums and streak three times at 60° by opening the lid of agar plates against flame in a semi- confluent lawn. Sterile paper discs (6 mm) (Himedia) saturated withextracts (20 µL) prepared in DMSO (having different concentrations) were aseptically placed on the upper layer of the inoculated nutrient agar and MacCONKEY agar surfaces, and plates were incubated at 37°C for 24 hour. Inhibition zones were appeared around the discs after 20-hour incubation which was recorded by zone reader in mm. Results were compared with standard antibiotic Cephalosporin. 20 µL DMSO in each discwas taken as a negative control. Results are described as mean ± standard deviation by performingAntimicrobial assay in triplicates.

2.4. Phytochemical screening

To identify the chemical constituents using standard method of Harborne and Kokate, stock solution of leaves of *Bauhinia variegata* was subjected with various chemical tests

3. RESULTS AND DISCUSSION

To evaluate the antibacterial activity of ethanolicleaf extract *Bauhinia variegata*, disc diffusion assay was chosen. Disc diffusion assay is more appropriate way to know the susceptibility of bacteria against a tested drug/ sample.

Antimicrobial assay determined that leaf extract of *B. variegata* is more productive against Gram (+ve) bacteria in comparison to Gram (–ve) bacteria. Maximum zone of inhibition recorded for*S. aureus* was 25mm at 0.8mg/mland minimum zone of inhibition was17mm at and 0.7mg/ml respectively illustrated in Figure. 1. Maximum zone of inhibition recorded for *S.typhi* was 17.5mm at 0.9 and 0.7 mg/ml and 14.5mm as minimum at 0.8mg/ml. In case of *Proteus vulgaris* maximum zone was 18.5mm at concentration of 0.8mg/ml and smaller inhibitory zone was 14mm at 0.6mg/ml. *Escherichia coli* was inhibited by 20mm zone of inhibition at 0.9mg/ml and minimum zone of inhibition 10.5mm was found at 0.6mg/ml (Table. 1). Our results depicted that considerable antibacterial activity against Gram(+ve) bacteria was examined in comparison to Gram (–ve) bacteria. The possible reason for this might be the presence of outer impermeable membrane, presence of periplasmic space, thin peptidoglycan single layer and cell wall composition in Gram negative bacteria due to which less or narrow activity was observed. Antibacterial activity of *B. variegata* was compared with standard drug Cephalosporin; pure antibiotic. It would be interesting to note that resistance shown by bacterial strains was remained independent to concentration of leaf extract. It means at some specific concentration more zone of inhibition was obtained as compared to its maximum concentration. Such irregular pattern was appeared in all bacterial strains except *E. coli*. *E. coli* only followed the traditional trend to some extend that increase in diameter of zone of inhibition with the increase of concentration of extract.

Extraction of plants can be carried out by different solvents like ethanol, acetone, methanol etc or sometimes these solvents are mixed with water to increase the extraction power. According a research, 20-95% ethanol/ water is recommended in extraction of herbal medicines [27]. In present studies for phytochemical analysis of *B. variegata* leaves 70% ethanol was mixed to 30% water to form ethanol extract. Leaf extraction of *B. variegata* revealed the occurrence of terpenoids, flavonoids, steroids glycosides, and anthraquinoneswhile phlobatannins andsaponins were not identified in tested plant sample (Table. 2). According to a reserche, alcoholic extract of *B. variegata* possessed alkaloids, oils and fats, glycosides, simple phenolics, tannins and saponins while terpenoids and flavonoids were absent [28]. This difference may depend on many factors such as geological source, soil condition, moisture content, drying method etc.

According to one more research degree of variability of antimicrobial activity is dependent on the presence of phytochemical constituents extracted by solvent [8]. Flavonoids aresynthesized in feedback of microbial infections by plants, and are hydroxylated phenolic metabolites [29]. Many plants contain nontoxic glycosides, phenolics were released by hydolyzing glycosides, which are lethal to microbial germs [30].antibacterial activity is also the property of terpenoids, exhibited by membrane integrity weakness and proton motive force dissipation [31]. Therefore, the presence of these phenolic compounds could justify the antibacterial activity observed in B. variegata.

Table 1: Representation of the antibacterial efficacy at different concentration of *Bauhinia variegata*'s leaves extract

Microorganisms	Bacterial strains	Concentration g/ml	Ethanolic extract DZOI(mm)	Standard	Control
Staphylococcus aureus	G +ve	0.9	24	39	-
		0.8	25		
		0.7	17		
		0.6	22		
Proteus vulgaris	G -ve	0.9	14.5	37.5	-
		0.8	18.5		
		0.7	16		
		0.6	14		
Escherichia coli	G –ve	0.9	20	36.5	-
		0.8	18.5		
		0.7	15.5		
		0.6	10.5		
Salmonella typhi	G -ve	0.9	17.5	25	-
		0.8	14.5		
		0.7	17.5		
		0.6	17		

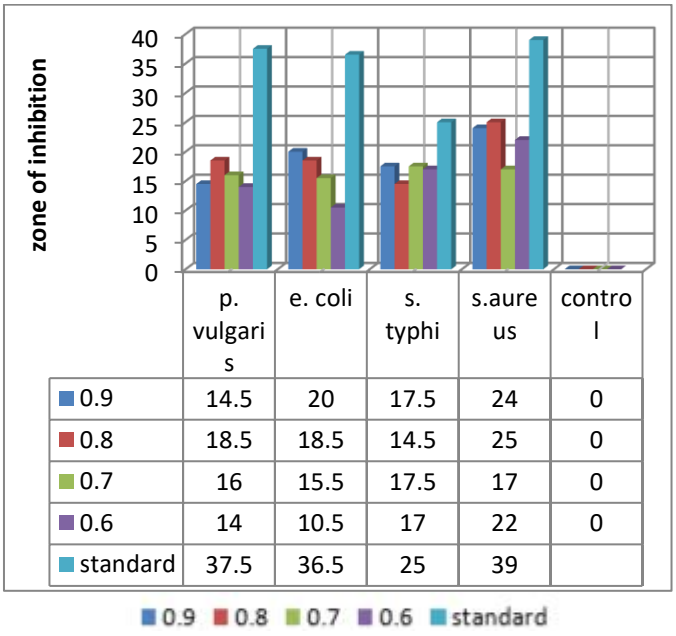

Figure 1: Graphical illustration is given to represent antibacterial activities by different strains of bacteria

Table 2: Phytochemical study of *Bauhinia variegata*'s leaves extract

Phytochemical constituents	Alcoholic leaves
Glycosides	Present
Terpenoids	Present
Flavonoids	Present
Steroids	Present
Phlobatannins	Absent
Saponins	Present
Tannins	Present
Anthraquinones	Present

4. CONCLUSION

Bauhinia variegata and other herbal medicines would be a substitute of antibiotic which are becoming nonfunctional now a day with the emergence of resistance in pathogenic bacteria. This study needs further elaboration by applying it on animal models/ specimens directly to demonstrate the effects of *Bauhinia variegata*.

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