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STUDY OF ANTI-BACTERIAL ACTIVITY OF BUDS OF *SYZYGIUM AROMATICUM*

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ABSTRACT

Antiacterial activity of acetone, chloroform and ethanol extracts of the buds of Syzygium aromaticum were studied against Bacillus subtilis and Esherichia coli by agar well diffusion method. The growth of both B. subtilis and E.coli were inhibited by all the three extracts of buds of Syzygium aromaticum.The present study has revealed that the antibacterial activity of these extracts against selected strains depends on the type of solvent used and its concentration for extraction and the bacterial stain tested. The result of the present study has revealed that all the buds extracts of Syzygium aromaticum have potent antibacterial activity when compared with conventionally used drugs and is almost equipotent to the standard (Amoxicillin) antibacterial drug. The drug may be further explored for its phytochemical profile to identify the active constituents responsible for antibacterial activity.

I. INTRODUCTION

The oldest form of health care known to mankind is herbal medicine. Herbalism also known as botanical medicine, herbology and phytotherapy is a traditional medicine practice based upon the use of plants and plants extracts. The scope of herbal medicine is sometimes extended to include fungal and bee product as well as minerals, shells and certain animal parts[1]. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are source of many potent and powerful drugs [2]. A wide range of medicinal plant parts (roots, stem, leaf, flower, buds, fruit, twigs etc.) extracts are used as raw drugs as they possess medicinal properties. Some of these raw drugs are collected in larger quantities by local communities and folk healers for local use while many drugs are collected in larger quantities and breeded to herbal industries as raw materials [3]. There are several reports on the antibacterial activity of different herbal extracts in different regions of world [4] but vast majority have not been adequately evaluated [5]

In older days, in search for rescue for their disease, the people looked for the drugs in nature. The beginning of the medicinal plants use were instinctive, as in the case with animals[6]In view of the fact that at the time there was no sufficient information either concerning the reason for the illness or concerning which plant and how it could be utilized as a cure, everything was based on the experience. In Ancient time, the reason for the usage of specific medicinal plants for treatment of certain diseases was being discovered thus, the medicinal plants usage gradually abandoned the empiric framework [7].

While the old people used medicinal plants primarily as simple pharmaceutical forms- infusions, decoctions and macerations. In the middle ages, particularly between 16th and 18th centuries, the demand for compound drugs

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was increased [8].The compound drugs comprised medicinal plants along with drugs of animal and plant origin. The drug compound as produced from a number of medicinal plants, rare animals, and minerals, it was highly valued and sold expensively [9].

Early 19th century was a turning point in the knowledge and use of medicinal plants. The discovery, substantiation and isolation of alkaloids from poppy (1806), quinine (1820), pomegranate (1878) and other plants then the isolation of glycosides marked the beginning of scientific pharmacy [10].Herbal medicine, also called botanical medicine or phyto medicine, refers to using a plant's seeds, berries, roots, leaves, bark or flowers for medicinal purposes [11].Plants have been used for medicinal purpose long before recorded history. Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while others developed traditional medicinal system (such as Ayurveda and Traditional Chinese Medicine) in which herbal therapies were used. Researchers found that people in different parts of the world tended to use the same or similar plants for the same purpose [12].

India has a rich heritage of traditional medicine which formed the basis of health care since earliest days of mankind. A large number of herbs or medicinal plant parts are used in several formulations for the treatment of many diseases caused by microbes. Herbal medicine is still the main stay of about 75-80% of the whole population, mainly in developing countries. The World Health Organization (WHO) estimated that almost 80% of the people worldwide rely on plant based medicines for their primary health care needs [13].and India happens to be the largest user of traditional medical cure, using 7000 plant species.

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [14].A wide range of medicinal plant parts (root, stem, leaf, flower, fruit, twigs, etc.) extracts are used as raw drugs as they possess many medicinal properties. Some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use while many raw drugs are collected in larger quantities and traded to herbal industries as raw material [15].There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world[16,17] but vast majority have not been adequately evaluated [18].

The increasing failure of chemotherapies and antibiotic resistance exhibited by pathogenic microbial infectious agents have led to the screening of several medicinal plants for their potential antimicrobial activity[19,20] Antibacterial properties of various plants parts have been well documented for some of the medicinal plants for the past two decades [21].

In India the herbal remedies is so popular that the government of India has created a separate department (AYUSH) under the Ministry of Health and Family Welfare. The National Medicinal Plants Board was also established in 2000 by the Indian government in order to deal with the herbal medicinal system [22]

Virulent strains of Gram negative bacterial *E.coli* can cause gastroenteritis, urinary tract infection and neonnatal meningitis. Some strains of *E.coli.* bacteria may also cause severe anemia or kidney failure, which can lead to death. Gram positive bacteria .*Bacillus subtilis* also known as the hay bacillus or grass bacillus is a gram positive bacteria found in soil and the gastrointestinal tract of ruminants and humans. They can contaminate food.

Plant *Syzygium aromaticum* is used to treat bacterial diseases, in treatment of inflammation, as an analgesic, as an oxidant, as an antiseptic, etc.[23,24]No work has been done on the antibacterial activity of the buds of *Syzygium aromaticum.* .Keeping in view the importance of of different types of infections caused by bacteria the

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present study has been carried out to find out the antibacterial potentiality of buds of *Syzygium aromaticum* against selected stains of bacteria.

II.MATERIALS AND METHDOS

2.1 Collection of Plant Material: The buds of *C were* purchased from the local herb shop of Patiala district of Punjab (India). The plant was identified, confirmed and authenticated[25].

2.2 Sample Preparation: The buds of *Syzygium aromaticum* was thoroughly washed and dried in hot air oven at 100°C for about 1hr. The dried sample was then grinded into fine powder using an electric grinder and stored in dessicator.

2.3 Extract Preparation: The extracts of buds of *Syzygium aromaticum* were prepared in ethanol, acetone and chloroform by following the methodology of Alam et.al[26]. 25g of finely grinded, dried bud powder was extracted using soxhlet apparatus, using 200ml of solvent ethanol, chloroform and acetone the extraction was done for about 24-36 hrs. at 30-35 0C, 25-300C, and 30-350C respectively. Solvents were removed under reduced pressure and the residues were collected and stored and further dried in vacuum dessicator over anhydrous calcium chloride to get a dry solid of extract for further study.

2.4 Phytochemical Analysis: The crude extracts were analysed for the presence of alkaloids, Tannins, saponins, trrpenoids, reducing sugars and flavonoids [27].

2.5 **Procurement of Microorganisms:**,*B. subtilis* and *E.coli* species were collected from department of Biotechnology and the pure cultures of bacteria were maintained on nutrient agar slants for their vegetative growth. The cultures were maintained in incubator for use and regularly checked for contamination, and the periodic transfers were made aseptically.

2.6 Culture of Test Microbes: For the cultivation of bacterial, Nutrient Agar Medium (Beef extract - 1.0 g, Yeast extract - 2.0 g, Peptone - 5.0 g, NaCl- 5.0 g, Agar - 15.0 g, distilled water 1 L) were prepared and sterilized at 15 lbs pressure and 121°C temperature for 25-30 min. Agar rest plates were prepared by pouring approximately 15ml of Nutrient Agar medium into the Petri dish under aseptic conditions.

2.7 Agar Well Diffusion Method: The ethanol, chloroform and acetonic extracts of buds of *Syzygium aromaticum* were tested by Agar Well Diffusion method4 mm holes were punched aseptically in nutrient agar plate by using a sterilized cork borer. The cotton swabs were dipped into the broth culture of the test organisms and were gently squeezed against the inside of the tube to remove excess fluid. *E.coli* and *B.subtilis* were swabbed on Agar plates. Swabbing was done in outside diameter of the plates. The plates were allowed to dry for about 5 minutes. Then the extracts of buds of *Syzygium aromaticum* in three solvents (ethanol, acetone and choloroform) with their different concentrations were added into wells of Petri plates. Pure solvents were used as control whereas amoxicillin was used as reference for bacterial species. The plates were incubated at 37°C for 24 hrs. The zones of inhibition were measured in millimeters (mm), using Vernier caliper. The zone size was recorded and all the cultures were discarded by autoclaving.

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Table 1: The observation of the Phytochemical tests of different extracts of the buds of *Syzygium aromaticum*

Test	Ethanol	Acetone	Chloroform
Alkaloids Wangersreagent (Reddish brown coloured ppts)	+	+	-
Carbohydrates Molish test (Reddish violet colour)	+	-	-
Terpenoids Conc. H2SO4 Test (Reddish brown color at interface)	+	+	+
Saponins Foam test (Presence of foam at surface)	–	–	–
Tannins Ferric Chloride test (Dark green colour)	+	–	+
Flavonoids Sodium Hydroxide test (Color changes from Yellow to colorless)	+	+	+

- absent; + present

Table 2: The zones of inhibition with different extracts of the buds of *Syzygium aromaticum*

Conc. of Extract	Test organism	Zone of inhibition		
		Ethanol Extract	Chloroform Extract	Acetone Extract
25%	*B. subtilis E. coli*	11mm 14 mm	11mm 10 mm	9 mm 6 mm
50%	*B. subtilis E. coli*	13mm 17 mm	12 mm 13 mm	10 mm 7 mm
75%	*B. subtilis E. coli*	16 mm 19 mm	15 mm 16 mm	12 mm 8 mm
100%	*B. subtilis E. coli*	19 mm 21 mm	16 mm 18 mm	14 mm 11 mm
Control	*B. subtilis E. coli*	NIL	NIL	NIL
Standard	*B. subtilis E. coli*	NIL	NIL	NIL
Amoxicillin	*B. subtilis E. coli*	25mm 30 mm	25mm 30 mm	25mm 30 mm

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III. RESULTS AND DISCUSSION

The ethanol, acetone and chloroform extract of buds of *Syzyzygium aromaticum* were tested for alkaloids, reducing sugars, saponins, tannins, terpenoids and flavonoids, and results are reported in table 1 and the results of zones of inhibition of these extracts with their different concentrations, control and(standard (amoxicillin) against the tested bacterial stains *B.subtilis* and *E.coli* are reported in table 2.

The zones of inhibition of solvent control were nil and of standard (amoxicillin) the zone of inhibition for *B. subtilis* and *E.coli* were 25 mm and 30mm respectively. The zones of inhibition observed for the difference extracts of buds of *Syzyzygium aromaticum* (table 2) at different concentrations were quite close to the zone of inhibition shown by standard (amoxicillin) for tested organisms. Thus the growth of both *B. subtilis* and *E.coli* were inhibited to a good extent by all extracts of. buds of *Syzygium aromaticum*

Therefore, it is recommended that extract and purification of bioactive compounds present in buds of *Syzygium aromaticum* are valuable in the preparation of drugs of different kinds. The assessments of various effects of such compounds on the animal and human health are required for future studies.

IV. CONCLUSION

The present study reveals the presence of many secondary metabolites in the seed extracts of. buds of *Syzygium aromaticum* It has also confirmed that the extracts of buds of *Syzygium aromaticum* could be used for the treatment of various infections. The bud extracts *Syzygium aromaticum* have potent antibacterial activity when compared with conventionally used drugs and is comparable to the standard (amoxicillin) antibacterial drug. The results lend credence to the folkloric use of buds of *Syzygium aromaticum* in treating bacterial infection and show that *Syzygium aromaticum* may be explored for its further phytochemical profile to identify the active constituents responsible for their use as potent antibacterial agents.

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