

Study of Antibacterial Activity of *Leaf Extracts of Dalbergia Sisso (Roxb.)*

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Abstract Antibacterial activity of ethanolic, distilled water and methanol extract of the leaves of *Dalbergia Sisso (Roxb.)* were studied against *Escherichia coli* and *Bacillus licheniformis* by agar well diffusion method. Results obtained showed that the growth of both *E.coli* and *B.licheniformis* were inhibited by all the three extracts of dried *Leaf Extracts of Dalbergia Sisso (Roxb.)*. The antibacterial activity of these extracts against selected bacterial stains depends on the type of solvent used for extraction. The present study revealed that *Leaf Extracts of Dalbergia Sisso (Roxb.)* can be exploited for new potent antibacterial agents.

Keywords: Dalbergia Sisso, World Health Organization

1. INTRODUCTION

Ancient time, 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. (Stojanoski, 1999). In view of the fact that at that 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 (Kelly, 2009).

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

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drugs was increased (Tpolak, 2005). The compound drugs comprised medicinal plants along with drugs of animal and plant origin. If the drug compound as produced from a number of medicinal plants, rare animals, and minerals, it was highly valued and sold expensively (Bojadziewski, 1992).

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 (Lukic, 1985). Herbal medicine, also called botanical medicine or phytomedicine, refers to using a plant's seeds, berries, roots, leaves, bark or flowers for medicinal purposes (Abeloff, 2008). 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 (Altschuler et. al., 2007).

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 (Famsworth, 1985) and India happens to be the largest user of traditional medical cure, using 7000 plant species.

Medicinal plants represents a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava et. al., 1996). 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 (Uniyal et. al., 2006). There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world (Parekh et. al., 2005), but vast majority have not been adequately evaluated (Balandrin et. al., 1985)..

The increasing failure of chemotherapies and antibiotic resistance exhibited by pathogenic microbial infections agents have led to the screening of several medicinal plants for their potential antimicrobial activity (Rich-Krc

et. al., 1996; Martins et. al., 2001). Antibacterial properties of various plants parts have been well documented for some of the medicinal plants for the past two decades (Leven et. al., 1979).

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 (Bottcher, 1965).

Virulent strains of Gram negative bacterial *E.coli* can cause gastroenteritis, urinary tract infection and neonatal meningitis. Some strains of *E.coli* bacterial may also cause severe anemia or kidney failure, which can lead to death (<http://www.m.webmd.com/>) Gram positive bacteria *B.licheniformis* is commonly associated with food spoilage and poisoning (Peopo et. al.,2003). Food poisoning by *B.licheniformis* is characterised by diarrhea and vomiting.

Dalbergia Sissoo Roxb. (Shisham, Sisoo, Tally) internationally premier timber species of the rosewood genus *Dalbergia*. *Sisoo* is reported a stimulant used in folk medicine and remedies (Oxford Dictionaries Online, 2014). It is used in conditions like emesis, ulcers, leucoderma, dysentery, stomach troubles and skin diseases (Ali, 2007). Pharmacological investigations indicated that its leaves posses different medicinal properties as antimicrobial (Mukhtar et. al., 2006), anti-inflammatory (Prabu et. al., 2006), antioxidant (Qjewale, 2005), antidiarrhoel (Majumdar et. al., 2005), antifertility (Ucendu and Leek, 1999), antiplamodial (Beldjoundi et. al., 2003), larvicidal and mosquito repellent activity (Ansari et.al., 2008).

But very little studies have been done on the antibacterial activity of plant extracts of *Dalbergia Sissoo (Roxb.)*. Keeping in view the importance of different types of infections caused by bacteria the present study was designed to find out the antibacterial potentiality of *Leaf Extracts of Dalbergia Sissoo (Roxb.)* against selected stains of bacteria.

2. MATERIALS AND METHDOS

2.1 Collection of Plant Material

The leaves of *Dalbergia Sissoo (Roxb.)* were purchased from the local herb shop of Patiala district of Punjab (India). The plant was identified, confirmed and authenticated (Jain, 1999).

2.2 Sample Preparation

The leaves of *Dalbergia Sissoo (Roxb.)* were 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.

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2.3 Extract Preparation

The extracts of the leaves of *Dalbergia Sisso (Roxb.)* were prepared in ethanol, distilled water and methanol by following the methodology of Alam et.al., 2010, 25g of finely grinded, dried root powder was extracted using soxhlet apparatus, using 150ml of solvent and the extract was done for about 36-48 hrs. at $25\pm 2^{\circ}\text{C}$. Solvent was removed under reduced pressure and the residues were collected and stored and further dried in vacuum desiccator 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, carbohydrates, proteins, steroid glycosides, polyphenolic compounds, saponine, tannins and flavonoids (Oloyede, 2009).

2.5 Procurement of Microorganisms

B.licheniformis 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, distilled water and methanol extract of leaves of seeds of *Dalbergia Sisso (Roxb.)* were tested by Agar Well Diffusion method (Alam et.al., 2010) (4 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.licheniformis* 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 leaves of *Dalbergia Sisso (Roxb.)* of concentrations (100%) were added into wells of Petri plates.

Pure solvents were used as control whereas gentamycin 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.

3. RESULTS AND DISCUSSION:

The ethanol, distilled water and methanol extract of *Leaf Extracts of Dalbergia Sisso (Roxb.)* were tested for alkaloids, steroid glycosides, saponins, tannins and flavonoids, and results are reported in table 1 and the results of zones of inhibition of these extracts with their 100% concentration and standard (gentamycin) against the tested bacterial stains *B.licheniformis* and *E.coli* are reported in table 2.

Table 1: The observation of the Phytochemical tests of different extracts of the *Leaf Extracts of Dalbergia Sisso (Roxb.)*

Test	Ethanol Extract	Distilled Water Extract	Methanol Extract
Alkaloids Wagner's reagent (Dark Brown coloured ppt)	—	+	+
Steroid glycosides Conc. H ₂ SO ₄ test (Reddish Brown color)	+	+	+
Saponins Foam test (Presence of foam at surface)	+	+	+
Tannins Ferric Chloride test (Dark Blue or Bluish Black product)	+	+	+
Flavonoids Sodium Hydroxide test (Appearance of Yellow color)	+	+	+
Glycosides Chloroform extract glacial acetic acid FeCl ₃ and H ₂ SO ₄ (Blue color appears in Acetic Acid Layer)	+	+	+

The zones of inhibition of solvent control were nil and of standard (gentamycin) the zone of inhibition for *B.licheniformis* and *E.coli* were 24mm and 22 mm respectively. The zones of inhibition observed for the difference extracts of *Leaf Extracts of Dalbergia Sisso (Roxb.)* (table 2) at 100%

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Table 2: The zones of inhibition with different extracts of the Leaf Extracts of *Dalbergia Sisso* (Roxb.)

Test organism	Solvent extract	Zone of inhibition	Control
<i>Bacillus Licheniformis</i> (<i>B.licheniformis</i>)	Ethanol	20 mm	—
	Distilled Water	—	—
	Methanol	18 mm	—
	Standard (Gentamycin)	24 mm	—
<i>Escherichia coli</i> (<i>E.coli</i>)	Ethanol	22 mm	—
	Distilled Water	15 mm	—
	Methanol	15 mm	—
	Standard (Gentamycin)	22 mm	—

concentration were quite close to the zone of inhibition shown by standard (gentamycin) for tested organisms. Thus the growth of both *B.licheniformis* and *E.coli* were inhibited to a good extent by all extracts of *Leaf Extracts of Dalbergia Sisso* (Roxb.).

Therefore, it is recommended that extract and purification of bioactive compounds present in *Dalbergia Sisso* (Roxb.) 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.

CONCLUSION

The present study reveals the presence of many secondary metabolites in the root extracts of *Dalbergia Sisso* (Roxb.). It has also confirmed that the root extracts of *Dalbergia Sisso* (Roxb.) could be used for the treatment of various infections. The root extracts of *Dalbergia Sisso* (Roxb.) have potent antibacterial activity when compared with conventionally used drugs and is almost equipotent to the standard (gentamycin) antibacterial drug. The results lend credence to the folkloric use of the root of *Dalbergia Sisso* (Roxb.) in treating bacterial infection and show that *Dalbergia Sisso* (Roxb.) 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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