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ANTIBACTERIAL ACTIVITY OF SEEDS OF *NIGELLA SATIVA* (KALONJI) BY AGAR WELL DIFFUSION METHOD

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ABSTRACT

Antibacterial activity of ethanolic, acetone and chloroform extract of the seeds of Nigella sativa (Kalonji) was studied against Escherichia coli and Bacillus licheniformis by agar well diffusion method. Results obtained showed that the growth of Nigella sativa(Kalonji). The antibacterial activity of these extracts against selected both E.coli and B.licheniformis were inhibited by all the three extracts of dried seeds of bacterial stains depends on the type of solvent used for extraction. The present study revealed that seeds of Nigella sativa can be exploited for new potent antibacterial agents.

Key words: Antibacterial, Escherichia coli, Bacillus licheniformis, Nigella sativa.

I. INTRODUCTION

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 [1]. 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 [2].

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 were increased [3]. 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 [4].

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 [5]. Herbal medicine, also called botanical medicine or phytomedicine, refers to using a plant's seeds, berries, roots, leaves, bark or flowers for medicinal purposes [6]. 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 [7].

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 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 [8]. 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 [9]. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world [10, 11], but vast majority have not been adequately evaluated [12].

The increasing failure of chemotherapies and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity [13, 14]. Antibacterial properties of various plants parts have been well documented for some of the medicinal plants for the past two decades [15].

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 [16].

Virulent strains of Gram negative bacterial *E.coli* can cause gastroenteritis, urinary tract infection and neomatal meningitis. Some strains of *E.coli.* bacteria may also cause severe anemia or kidney failure, which can lead to death. Gram positive bacteria *B.licheniformis* is commonly associated with food spoilage and poisoning [17]. Food poisoning by *B.licheniformis* is characterized by diarrhea and vomiting.

Nigella sativa (Kalonji) called "Black Cumin" is an annual flowering herb in the ranunculaceae family, native to South and South West Asia, also known as " fennel flower plant". *Nigella sativa* plant has finely divided blue and foliage flowers which produce black seeds, cultivated extremely around India, Pakistan, Mediterranean countries, South Europe, Syria, Turkey, Saudi Arabia [18]. The seeds of kalonji have been used in mercury poisoning, sores and leprosy [19]. Seeds of *Nigella sativa* are known to exhibit anticestodial and antinematodal activity, [20, 21] anticancer activity [22], antidiabetic activity [23], antiulcer activity [24-26] and antimalarial activity [27]. But very little studies have been done on the antibacterial activity of plant extracts of *Nigella sativa* (Kalonji). 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 seeds of *Nigella sativa* against selected stains of bacteria.

II. MATERIALS AND METHDOS

2.1 Collection of Plant Material: The seeds of *Nigella sativa were* purchased from the local herb shop of Patiala district of Punjab (India). The plant was identified, confirmed and authenticated [28].

2.2 Sample Preparation: The seeds of *Nigella sativa* 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.

2.3 Extract Preparation: The extracts of the seedsof the *Nigella sativa* were prepared in ethanol, acetone and chloroform by following the methodology of Alam et.al 25g of finely grinded, dried seed powder was extracted using soxhlet apparatus, using 150ml of solvent and the extract was done for about 36-48 hrs. at 25±2°C. Solvent was removed under reduced pressure and the residues were collected and stored and further dried in vacuum desicator 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, Tannis, steroids, saponins, Phlobatannis and flavonoids [29].

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, chloroform and acetonic extracts of seeds of *Nigella stiva* were tested by Agar Well Diffusion method [30]. 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 seeds of *Nigella sativa* in three solvents (ethanol, acetone and choloroform) with their 100% concentration 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.

Table 1: The observation of the Phytochemical tests of different extracts of the seeds of *Nigella stiva* (Kalonji)

Test	Ethanol	Acetone	Chloroform
Alkaloids Mayer's reagent (Cream coloured ppts)	+	-	-
Carbohydrates Fehling solution test (Yellow or red colour ppts)	+	-	-
Proteins Ninhydrin reagent (Violet color)	+	+	+
Steroid glycosides Conc. H_2SO_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)	+	+	+
Phylobotannins 1% HCl Test (Red colored ppts.)	-	-	-

Table 2: The zones of inhibition with different extracts of the seeds of *Nigella stiva*(Kalonji)

Test organism	Solvent extract	Zone of inhibition	Control
Bacilus Licheniformis (B.licheniformis)	Ethanol	19mm	—
	Acetone	23 mm	—
	Chloroform	18mm	—
	Standard (Gentamycin)	24 mm	—
Escherichia coli (E.coli)	Ethanol	18 mm	—
	Acetone	21mm	—
	Chloroform	15mm	—
	Standard (Gentamycin)	22 mm	—

III. RESULTS AND DISCUSSION

The ethanol, acetone and chloroform extract of seeds of *Nigella sativa* were tested for alkaloids, carbohydrates, proteins, steroids, saponins, tannins, Phylobotannins 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.

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 seeds of *Nigella sativa*(table 2) at 100% 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 seeds of *Nigella sativa*.

Therefore, it is recommended that extract and purification of bioactive compounds present in *Nigella sativa* 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 *Nigella sativa*. It has also confirmed that the seed extracts of *Nigella sativa* could be used for the treatment of various infections. The seed extracts of *Nigella sativa* 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 seed of *Nigella sativa* in treating bacterial infection and show that *Nigella sativa* 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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