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Research Article

Phytochemical Analysis and Antimicrobial Activity of *Punica granatum* L. and *Zingiber officinales* L. Plant Extracts against Microbes Causing Food Poison

Shweta J Sabannavar*

Assistant Professor in Botany, Department of Botany, P C Jabin Science College, Autonomous, Hubballi, Karnataka, India

***Corresponding Author:** Shweta J Sabannavar, Assistant Professor in Botany, Department of Botany, P C Jabin Science College, Autonomous, Hubballi, Karnataka, India.

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Abstract

Food poisoning brings illness and side effects to human beings, to prevent pathogen related infections people use chemical preservatives. These chemical substances have a negative effect on human health because of such concerns we need to find very effective, safer and natural alternative preservatives. In the present study phytochemical screening and antimicrobial activities of *Punica granatum* L. and *Zingiber officinale* L. was evaluated. Plant extract have been used to control food poisoning pathogen and preservation. Qualitative preliminary phytochemical screening of *P. granatum* peel extracts and ginger were assessed by standard methods. The results revealed that activity of secondary metabolites in ethanol and acetone is higher than chloroform and petroleum ether. The antimicrobial activity of pomegranate and Ginger was analyzed against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Aspergillus niger* using disc and well diffusion methods. The ethanol and acetone extract of the two plants were prepared and results revealed that concentration of extract effect the activity of microorganism and *P. granatum* showed high antimicrobial activity than ginger. These plants extracts can be used as a natural preservative to control food poisoning, fungicides and used as a food preservative. It can be used as healthy molecules in diet chart. The natural selection of preservatives decreases the rate of human health hazards and acquisition of microbial resistant.

Keywords: Antimicrobial Activity; Disc Diffusion; Well Diffusion; Phytochemical Screening; Preservative

Introduction

Food is a source of carbohydrate, fats, protein and other minor and major compound which is required for growth, reproduction and for many other biological activities of the body. The food preservatives used to preserve food and involve in reduction of growth cycle of the microorganisms such as bacteria, yeast, mold, virus to prevent the food spoilage. Lack of provision and transportation is one of the reasons behind food shortage in developing countries. Certain area has a transient surplus of food products while other area has a deficiency. There could be many factors affect the function of microorganism in food, they may have a useful function or harmful function. The significance of microorganism in foods rely on number of factors like the quantity and type of microorganisms, food processing and storage treatments. The microbial decay of food usually responsible for organoleptic changes like change in appearance, odor, slime formation, texture and taste. The agents which can be responsible for foodborne illness are microorganisms, parasites, chemicals, and metabolic disorders [12].

Food preservatives that are added to food, normally in small quantities to enhance the appearance, texture, taste, shelf life or other storage properties. Bacterial spores are considered as a most resistant type of microorganisms and fungal spores are more re-

sistant than vegetative cells to the action of preservatives, higher the microbial load greater is the amount of chemical preservatives needed to inhibit the microbial population. Microorganism are most important agents of foodborne illness. Only small numbers of organisms are required to cause infection. Microorganisms have the capacity for rapid genetic change and adaptation which can undergo changes that lead to problems. They can adapt to new environmental situation and become more pathogenic and responsible for human health hazard.

Acrylonitrile and Vinyl chloride are carcinogens and used in the manufacture of food packaging material and very minute quantity of this carcinogens appear in food. Such chemical substance is not considered as a food additive by the Food and Drug Administration (FDA). There are numbers of carcinogens present in food as natural constituents, additives or contaminants. The toxicity of chemical to humans may be due to the same mechanism that alter the microorganism or the person to develop a hypersensitivity to the compound. This results in an allergic reaction. As the dominant organism grows it can cause changes in the food and makes the food more suitable for the other pathogenic organisms. The repeated application of chemical preservatives has resulted in the accumulation of chemical residue in food chain and food products, accession of microbial resistance, allergies and side effects of these substance on human health [5].

Nitrates, Sulfites, Nitrites and benzoates are group of common chemical preservatives used in various food and food products such as tea, fruit juice and coffee now excessive use of this chemicals may have side effect and cause allergies, palpitation, asthma, brain damage and skin rashes. In ancient time several herbs and spices were used in food, not only as a flavoring agent and food preservative (salt, sugar, vinegar, honey) but also as a traditional medicine [2]. Medicinal plants are rich secondary metabolite like, alkaloids, flavonoids, tannins, glycoside and terpenoids. These substances have been reported to have broad range of medicinal value. These plant extracts considered as natural sources of antimicrobial agents, regarded as nutritionally safe and easily degradable [3]. The use of natural bioactive compounds, functional microbial starter cultures and antioxidants for "synthetic preservative-free" products are included among the latest and most successful accomplishments in the food industry [13].

The *Punica granatum* L. is a slow growing, small, spiny and deep rooted multipurpose deciduous small tree. It can reach a height up to 5 to 10 meters. Pomegranate is a good source of secondary metabolites specially tannins and has many applications in agroforestry, pharmaceutical, food industry for its medicinal properties and it is also grown as an ornamental plant. Pomegranate cultivated in temperate and subtropical zones. Pomegranate has been used extensively in the folk medicine. Pomegranate has been used to treat cough, sore throat, digestive disorders, arthritis, skin disorders, skin cancer, diabetes, prostate cancer, osteoarthritis and to remove tapeworms. Study showed that pomegranate seeds used in treatment of digestive system of fats. Consumption of pomegranate help to increase blood flow and reduce blood pressure and plaque in arteries thus, prevent strokes and heart disease. In Iran and Saudi Arabia, pomegranate fruit juice is a very popular beverage and often converted into wine. In South California, pomegranate used to make jelly by adding sugar and liquid pectin.

The *Zingiber officinale* L. is an herbaceous perennial, rhizomatous plant of the family *Zingiberaceae*, used as a spice, food, flavoring agent. Quality and quantity of ginger depend on type of soil, disease management and irrigation facility. Ginger is an important commercially grown aromatic crop which are used as a medicine, spice, condiment and act as anti-inflammatory ginger and its preparations are very well accepted mainly in pharmaceutical for its anti-inflammatory, cytotoxic, antioxidant, antitumor, antibacterial, antidiabetic, antiviral, antihelmintic and antifungal activities [14]. It is also used to treat arthritis, digestive problems, heart and circulatory problem, sinus congestion, nausea, migraine and fever. The microbial infections are the major source of mortality and morbidity of developed and developing country. Number of commercially available antibiotics used for treatment and management but misuse of this antibiotic develops antibiotic resistance and responsible for human health hazards. Therefore, it is necessary to find new and safe bioactive compound.

Objective of the Study

The main objectives are to investigate the phytochemicals compounds, anti-microbial activity using Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis)*, Gram negative bacteria (*Escherichia coli*) and fungi (*Aspergillus niger)*.

Materials and Methods

The fresh plant material of two plant species *Punica granatum* L. and *Zingiber officinale* L. were collected from local market of Bengaluru, India. The collected plants were washed with distilled water and 20- 30 % ethanol and dried in vacuum chamber. The dried plant material of pomegranate and ginger was grounded into fine powder until fracture was uniform and smooth.

Plant extraction

About 25 g of the powder sample was soaked in 100 ml of ethanol and Acetone with stirring for 48 h. filtered the sample and centrifuge at 9000 rpm for 10 minutes and filtered again through Whatmann filter paper to attain clear filtrate. The filtrates were dried at 40°C using rotatory vacuum evaporator, remaining extract yields were weighted and stored in a small bottle in fridge at 5°C.

Collection of microbial samples

The plant extracts were tested for Antibacterial activity against the bacterial strain such as *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis* were obtained from Veterinary College, Bengaluru and fungi sample of *Aspergillus niger* were isolated from onion and maintained at the Botany laboratory of Mount Carmel College Autonomous, Bengaluru to use as the test organism for study. Ethanol and Acetone extract of pomegranate and ginger were tested against the above organisms.

Antibacterial activity of plant extracts

In vitro Antibacterial activities were evaluate using different plant extracts against Gram positive and Gram-negative pathogenic bacteria using agar disc diffusion method using sterilized forceps. The plant extract residues were re-dissolved in 2.5 ml of ethanol and acetone then loaded over sterile filter paper discs to obtain final concentration of 10 mg/disc. 24 hours culture of bacterial were streaked on the Muller- Hinton agar and allowed to grow at 37°C for 3-4 hours. Sterile filter paper discs loaded with plant extract concentration of were placed on the top of Mueller-Hilton agar plates and Tetracycline disc were used as a control for antibacterial activity. The zone of inhibition around the disc was measured after 24 hours of incubation at 37°C recorded and considered as indication for antibacterial activity [8].

Antifungal activity of plant extracts

In vitro Antifungal activities were evaluate using different plant extracts against *Aspergillus niger* using well diffusion method [3] with sterile cork borer. The culture of 48 hours old grown on Rose Bengal Agar (RBA) were used for inoculation of fungal strain on Sabouraud Dextrose Agar (SDA) plates. Suspension of fungal spore were prepared in sterile Phosphate buffered saline (PBS). An aliquot (0.1 ml) of inoculum was introduced to SDA and poured in to a Petri dish by pour plate method. After solidification, the appropriate wells were made on SDA plate by using sterile cork borer. In agar well, diffusion method 0.1 ml of Ethanolic and Acetone extract of pomegranate and ginger were introduced. The zone of inhibition around the disc was measured after 24 hours of incubation at 37°C recorded and considered as indication for antifungal activity.

Preparation of extraction for preliminary phytochemical screening

The collected plants were washed with distilled water and dried in shade. The dried plant material of pomegranate and ginger was grounded into fine powder until fracture was uniform and smooth. The powdered drug (1g) was suspended in 50 ml of the solvent (Ethanol, Acetone, Chloroform, Petroleum ether) in a beaker with stirring for 10 hours. The supernatant was filtered by using Whatmann Filter paper. The filtrates were dried at 40 °C using rotatory vacuum evaporator to obtain crude extract.

Phytochemical screening

Phytochemical screening of *Punica granatum* L. peel extract and *Zingiber officinale* L. rhizome extracts were analyzed for alkaloids, Coumarin, flavonoids, Phenols, Quinone, Saponin, Tannins, Anthocyanin, Steroids, Terpenoids, Cardiac glycosides by standard method [9,11].

Results

Phytochemical analysis of plant sample

The ethanolic peel extract of *Punica granatum* L. showed the presence of all phytochemical compound except glycoside, Anthocyanin and tannins. The chloroform peel extract showed only presence of tannins, saponins, phenol, Quinines, terpenoids and steroids. Petroleum ether extract of *Punica granatum* L. peel showed the presence of saponins and phenols. Acetone extract of *Punica granatum* L. showed the presence of all phytochemical constituents except saponins, anthocyanins and alkaloids.

The ethanoic peel extract of Ginger showed the presence of all phytochemical compound except Tannins, Flavonoids, phenols, alkaloids and anthocyanins. The chloroform extract showed only presence of tannins, saponins and flavonoids. Petroleum ether ex-

tract of Ginger showed presence of saponins, steroids. Flavonoids and anthocyanins. Acetone extract of Ginger showed presence of saponins, glycosides, flavonoids, steroids, coumarin and terpenoids.

Antimicrobial activity of plant extracts

Two plant species were studied to evaluate their antibacterial and antifungal activity against food poisoning microorganisms including two strains of gram-positive bacteria *B. subtilis* and *S. aureus* and one strain of gram-negative bacteria *E. coli* using disc diffusion method and agar well diffusion method. The results revealed that both plant extracts were effective in suppressing microbial growth of food poisoning bacteria with variable potency. Pomegranate was the most effective extract retarding microbial growth of all tested pathogenic bacteria at concentration of 10 mg/ml while extract of ginger showed variable antimicrobial activity against food poisoning bacterial strains. Pomegranate extract were the most effective extracts and showed a strong antimicrobial activity against food poisoning microbes.

In general, gram-negative bacteria is less sensitive than gram positive bacteria. The reason for less sensitivity of the gram-negative bacteria than gram positive bacteria could be for their differences in cell membrane compounds and arrangements or their outer phospholipid membrane and porins constitute. In this study we found that *E. coli* was more resistant to all ginger extract.

Test	Chloroform	Petroleum ether	Ethanol	Acetone
Tannins	+	-	-	-
Saponins	+	+	+	+
Quinines	+	-	+	+
Terpenoids	+	-	+	+
Steroids	+	-	+	+
Flavonoids	-	-	+	+
Phenols	+	+	+	+
Alkaloids	-	-	+	-
Glycosides	-	-	-	+
Coumarins	-	-	+	+
Anthocyanin	-	-	-	-

Table 1: Showing preliminary phytochemical analysis of pomegranate.

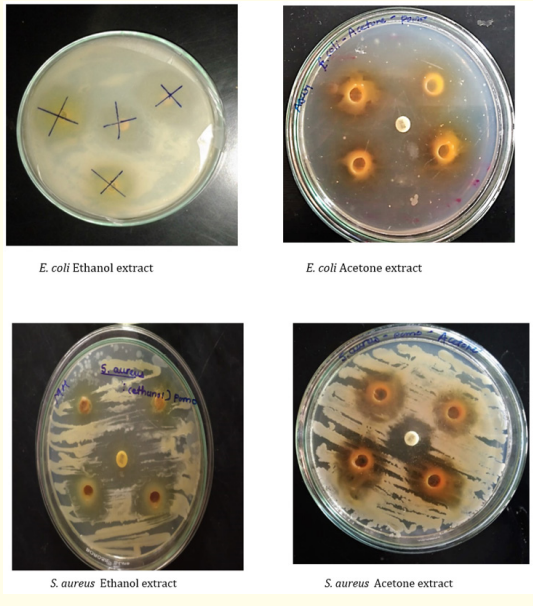
Test	Chloroform	Petroleum ether	Ethanol	Acetone
Tannins	+	-	-	-
Saponins	+	+	+	+
Quinones	-	-	+	-
Terpenoids	-	-	+	+
Steroids	-	+	+	+
Flavonoids	+	+	-	-
Phenols	-	-	-	-
Alkaloids	-	-	-	-
Glycoside	-	-	+	+
Coumarins	-	-	+	+
Anthocyanin	-	+	-	-

Table 2: Showing preliminary phytochemical analysis of ginger.

Test organism	Zone of inhibition (mm)			
	Punica granatum L.		*Zingiber officinale* L.	
	Ethanol	Acetone	Ethanol	Acetone
Escherichia coli	24 ± 0.75	25 ± 0.40	7 ± 0.59	8 ± 0.45
Staphylococcus aureus	23 ± 0.48	24 ± 0.40	14 ± 0.34	10 ± 0.48
Bacillus subtilis	23 ± 0.45	22 ± 0.34	15 ± 0.35	9 ± 0.28
Aspergillus niger	25 ± 0.45	30 ± 0.34	18 ± 0.51	10 ± 0.55

Table 3: Showing antimicrobial activity of pomegranate and ginger plant extract.

The values are the Mean standard error.



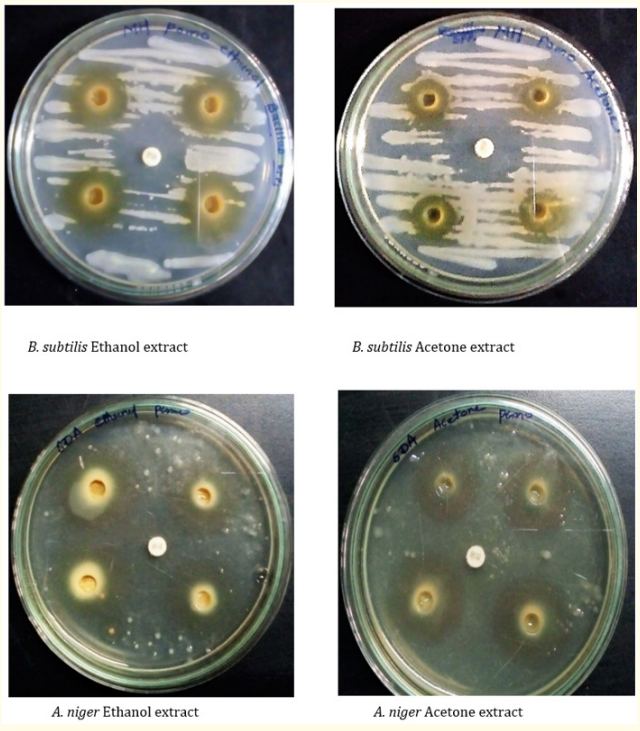


Figure 1: Zone of inhibition of Pomegranate plant extract.

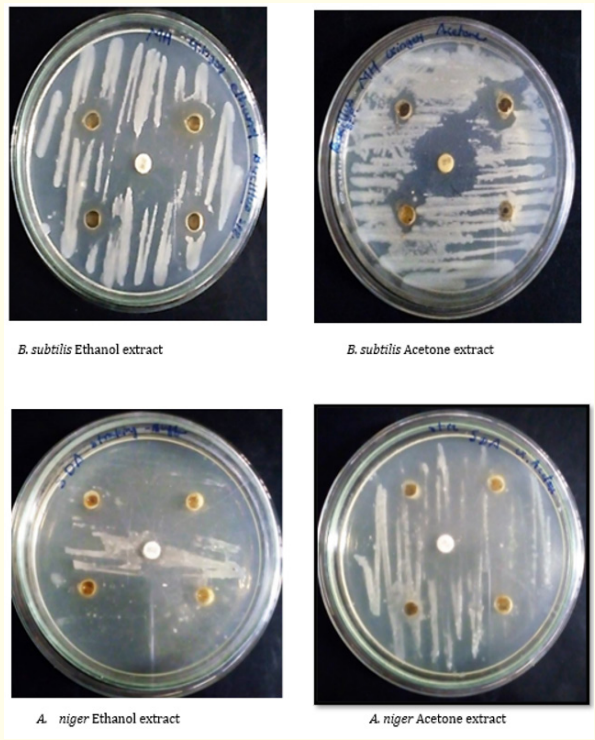
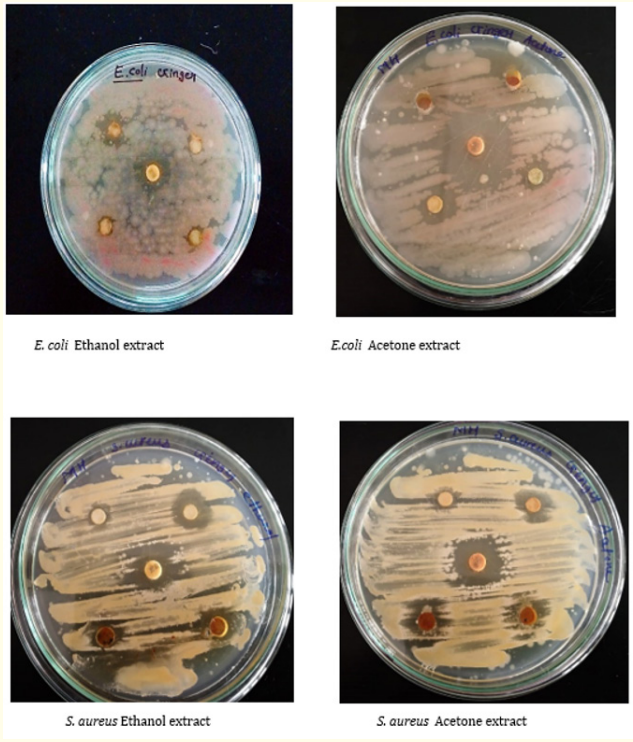


Figure 2: Zone of inhibition of Ginger plant extract.



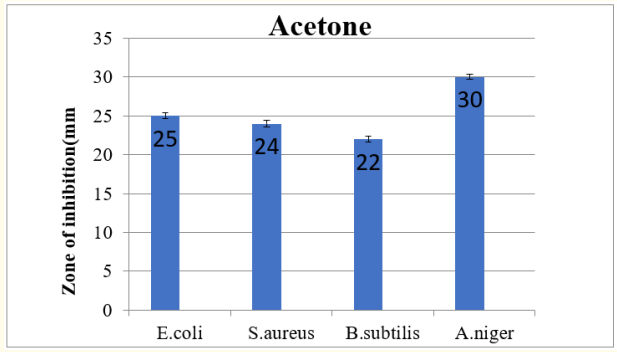
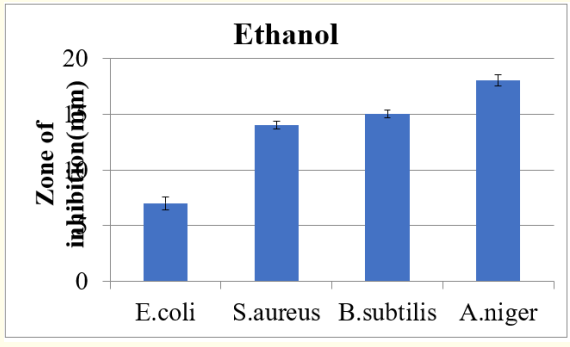


Figure 3: Antimicrobial activity of Pomegranate.



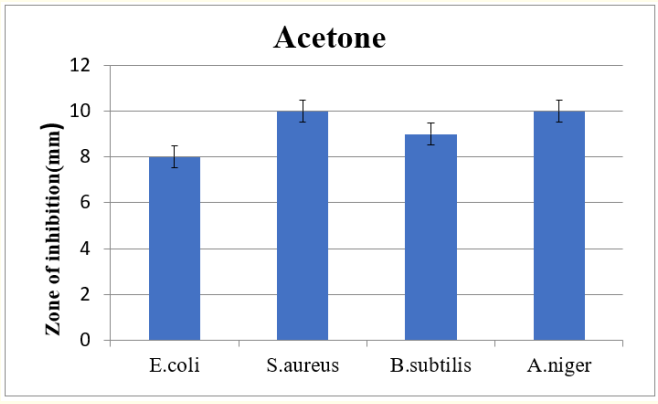


Figure 4: Antimicrobial activity of Ginger.

Discussion

Medicinal plants are normally used for its antimicrobial properties to control and prevent diseases. This plants product receives major interest due to their multi drug resistance activity. *Punica granatum* L. is a significant source of hydrolysable tannins, anthocyanins, gallic acid and ellagic acid [6] and Vitamin C. Plants, herbs, spices and vegetables used in traditional medicine have been accepted currently as one of the main source of chemo preventive drug discovery and development. Study indicates that many plants polyphenols such as catechins, chlorogenic and ferulic acid act as important anti-carcinogenic, antioxidant and antimitotic agents.

In general, gram-negative bacteria is less sensitive than gram-positive bacteria, the reason for less sensitivity of the gram-negative bacteria than gram-positive bacteria could be for their differences in cell membrane compounds and arrangements or their outer phospholipid membrane and porins constitute. In this study we found that *E. coli* was more resistant to all ginger extract. The reason for higher sensitivity of the gram-positive bacteria than gram-negative bacteria could be ascribed to their differences in cell membrane constituents and their arrangement and that the Gram-positive bacteria contain, an outer peptidoglycan layer, which is an ineffective permeability barrier. The present studies demonstrated that the bioactive principles are responsible for the antimicrobial activities against bacterial and fungi species could be further use in recognizing and isolating the structure to form remedies against these microorganisms. The result showed the great antimicrobial activity of ginger and pomegranate peel extract against the tested food borne pathogens- *Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Aspergillus niger.* Antimicrobial activity of crude ginger at room temperature and boiling temperature [10] and results revealed that boiling temperature treated crude sample of ginger lost its antibacterial property against *Escherichia coli, Klebsiella peumoniae* and *Staphylococcus aureus* but Antimicrobial activity of ginger at room temperature and boiling temperature [15] and results revealed that the extraction of ginger in soybean broth protected the antibacterial activity of ginger at boiling or high temperature. The synergistic effect of ethanol extract of ginger against *Staphylococcus aureus* and *Bacillus* spp [16].

Plants are known to contain numerous biological active compound which possesses curative properties. Secondary metabolites afford exigent pharmaceutical properties for human health. Compound belonging to the alkaloids, flavonoids and terpenoids, are used as dietary supplements or as drugs to heal or prevent various diseases and in particular some of these compounds seem to be competent in preventing and inhibiting various types of cancer [1]. Effect of pomegranate peel extract on microorganism such as *Salmonella enterica, Bacillus subtilis, Shigella sonnei, Staphylococcus aureus, Enterococcus faecalis* and *Escherichia coli* is controlled by different concentration of plant extract [17]. The antimicrobial activity of tropical plants using disc diffusion method against, *Staphylococcus aureus* and *Escherichia coli.* From the study they demonstrated that *Punica granatum* L. extract produced 11 - 20 mm size of zone of inhibition in the present study inhibition zone size is more than 20 mm [7].

Most of the people involved in the manufacture of preparation of herbal drugs lack the basic knowledge of the drugs. Consequently, adulteration or substitution of plants in place of original ones permeate the pharmaceutical industries, rendering the herbal drugs undependable and invalid. This leads to unpopularity of phyto-drugs among the people. Therefore, it is necessary that all medicinal plants should be subjected through botanical standardization. The phytochemical screening of the drug is a very important aspect in the process of standardization and quality control because the constituents vary qualitatively and quantitatively not only from plant to plant, but also different samples of the same species. presence or absence of certain compounds in an extract is determined by the color reaction of the compounds with the specific chemical which act as dyes. This procedure is a simple preliminary pre-requisite before going for the detailed phytochemical investigation. Various tests have been conducted qualitatively to find out the presence and absence of bioactive compounds. Phytochemical screening will provide a general idea regarding the presence of secondary active compounds. This research has provided insight on the use of secondary metabolites in traditional medicine in maintaining proper health.

Personal preferences ethnic issue or origin and family background may play a role in an individual deciding whether a food is spoiled. The economic costs associated with this type of disease are enormous and the controversies surrounding the safety of foods have had a major technical and economic impact on the agricultural and food industries

Conclusion

A safe, inexpensive, effective and ecofriendly system is needed to control the human health hazards. The results obtained from antimicrobial activity of pomegranate and ginger was that it can be used as an antimicrobial agent in pharmaceuticals. There is wide body of scientific study and evidence to show that pomegranate and ginger has great potential in the treatment of many microbial diseases.

It is important to isolate this secondary active constituent and determine their toxicity and pharmaco-kinetic properties to treat various disease and for crop improvement. Results revealed that different plant concentration affect the growth of different microbe so, standardization of extraction process is very important aspect. Secondary active constituent of Pomegranate and Ginger inhibit the growth of *Aspergillus niger*. Isolation of this compound and commercial application replace the chemical or synthetic fungicides used in agriculture. *In vitro* evaluation of this microorganism to test the efficacy in controlling the incidence of disease in crops, plants, and humans. Bioactive principles are responsible for the antifungal and antibacterial activities against different kind of micro-organism could be further used in recognizing and isolating the structure and to formulate the remedies against these microorganisms.

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