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PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF SELECTED SPICES AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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Abstract:

In order to enhance the flavour of food, spices are defined as fragrant or tasty plant components, whether they are of indigenous or foreign origin. Herbs and spices were used in the middle times for seasoning, food preservation, and medicinal purposes. The goal of this study was to evaluate and compare six different commercially available spices that are widely used in the production of ready meals in terms of their phytochemical and antibacterial properties. The extract from these species was found to contain a variety of phytochemicals, such as saponins, tannins, flavonoids, terpenoids, etc. after being put through phytochemical screening assays. Since they are a key source of antimicrobial compounds to fight infections, their extracts were tested using the well diffusion method for their antibacterial activity. The findings suggested that the extracts might be antibacterial for the species under investigation. The inhibitory zone of the ethanol-prepared garlic extract is larger than that of the nine-spice extract that was tested. In an agar well diffusion assay for antimicrobial activity, garlic extract had an inhibitory zone with a diameter of 13.45 mm, compared to a diameter of 7.15 mm for black cumin, 12.50 mm for ginger, 10.30 mm for cloves, and 7.15 mm for 10.30 mm for clove extract. According to the study's findings, spices may be useful in the hunt for antibiotics because they contain anti-microbial characteristics against Staphylococcus aureus infections (diseases).

Keywords: Spices, S. aureus, K. pneumonia, E. coli, Antibacterial, Phytochemical

1. Introduction

Many modern medications have been isolated from natural sources, which has been a source of medicinal agents for thousands of years. Many of these isolations were based on the applications of the agents in traditional medicine. With nearly 80% of the world's population relying mostly on traditional medicines for their primary health care, this plant-based traditional medicine system continues to play a crucial role in healthcare [1,2]. These plants' therapeutic properties come from phytochemical components that have distinct pharmacological effects on the human body. Many therapeutically helpful medications for the treatment of human ailments have been discovered as a result of the quest for new pharmacologically active molecules derived by screening natural resources like plant extracts. Alkaloids, tannin, flavonoid, and phenolic compounds are the most significant of these bioactive components found in plants [3,4]. These plants include term "spice" refers to a variety of plant materials, including leaves (coriander, mint), buds (clove), bulbs (garlic, onion), fruits (red chilli, black pepper), stems (cinnamon), rhizomes (ginger), and other plant parts, that are aromatic or have a strong flavour and are used to enhance food flavour. Herbs are plants with flavorful leaves and

are referred to as a subset of spices [5]. Herbs and spices have had a significant impact on civilization and the history of nations. Spices are essential in the creation of delectable dishes due to their flavour and pungency [6].

They are also said to have a variety of medical and pharmacological effects, particularly antibacterial action against various germ types [7–12]. Spices have long been thought to have digestive stimulating effects in addition to improving food flavour and taste [13]. The prevailing consensus is that spices enhance gastric juice output and salivary flow, which helps with digestion [14]. A number of pharmaceutical formulations are used, in addition to other plant materials, to treat digestive issues [15]. Spices have long been known to provide a few therapeutic benefits, including tonic, carminative, stomachic, diuretic, and antispasmodic effects [16–18].

They have gained pharmacological applications in the indigenous systems of medicine as digestive stimulants and to treat digestive diseases due to these characteristics, which are primarily empirical but nevertheless effective [13]. Humans have employed plant extracts and natural substances that have been refined from plants for many millennia to treat and relieve a range of inflammation-related disorders, including cancer [19]. Spice-derived phytochemicals hold great promise for both cancer prevention and treatment [20–22]. They can trigger apoptosis, stop tumour cell growth, thwart invasion and angiogenesis, and even stop bone resorption. These phytochemicals have a variety of targets through which they exert their actions, yet pharmacologically, they are extremely safe [20].

Plants contain natural bioactive substances called phytochemicals. The two categories of phytochemicals are primary compounds and secondary compounds. According to their roles in plant metabolism, these classes have been established. Primary chemicals include amino acids, carbohydrates, proteins, and chlorophyll, while secondary compounds include things like terpenoids, phenolic compounds, and alkaloids [23]. Although plants create these molecules to defend themselves, new study has shown that several phytochemicals can also shield people from disease. Fruits and plants contain a variety of phytochemicals, and they all function in unique ways.

Numerous plant extracts have been demonstrated to prevent the development of germs. These chemical-rich extracts are typically thought to aid plants in mounting a defence against infections from pathogenic microbes [24]. Spices and herbs are thought to be the natural chemical component that gives them their antibacterial actions against some diseases in addition to their protective properties. Spices and herbs are proven to be effective against both gram-positive and gram-negative bacteria, including Escherichia coli, Salmonella, Staphylococcus, and Streptococcus species, according to numerous studies. In order to investigate plants' antibacterial activity, screening and effectiveness testing are conducted [25,26].

The demand for alternative antimicrobial agents has sparked interest in researching plant extracts with proven therapeutic properties for use in the production of herbal antimicrobial agents by pharmaceutical companies. The need to find new antimicrobial agents has grown as a result of the growth of microorganism resistance to several synthetic antimicrobial agents that are commercially produced. The majority of bacterial and fungal infections have demonstrated the capacity to evolve resistance to some of the currently marketed antimicrobial drugs. This research aims to identify the phytochemical characteristics and antimicrobial activity of a few spices and herbs and to evaluate whether these extracts have antibacterial tendencies against bacteria infections.

2. Methodology

Preparation of Extracts: The spices, Clove, Garlic, Black pepper, Bay leaf, that were mature, healthy, and fresh were gathered and cleaned with distilled water. The unwanted parts were then removed using a sterile knife, once the fleshy part of garlic had properly dried. Then, the dried sample was grinded

into powder. The powdered samples were then mixed into 95% ethanol and chloroform, the extraction of secondary metabolities was carried using Soxhlet apparatus [12].

Physicochemical Screening:

Terpenoid:	1gm extract was mixed with 2ml chloroform and concentrated H_2SO_4.
Flavonoid:	1ml extract was mixed with NaOH and HCl.
Phlobatanin:	1 millilitre of extraction was mixed in purified water & processed. In a 2 percent HCl, the filtrate was diluted.
Tannins:	1% lead acetate was added in 1ml extract.
Coumarin:	In 2ml of extraction, 3ml of 10% NaOH was applied
Steroid:	1 mL extraction was mixed in 10 mL chloroform, and an appropriate amount of sulphuric Acid was applied to the test tube's sides.
Saponin:	One millilitre of extraction was combined with three millilitres of dw and allowed for shaken vigorously.

Antibacterial Activity of Spices: Nutrient agar media (20 ml per Petri plate) were prepared. A sterile borer was used to create 8mm wells after 20µl of microbial cultures had been spread. In the event that there were bacteria, 50µl of sample was placed into wells and cultured for 48 hours at 37ºC. A ZOI (Zone of Inhibition) calculation was made.

Statistical Analysis: The assessment of the general association of the plant species as well as fractions of the extracts was done using a non-parametric one-way ANOVA analysis. The threshold for statistical significance was 0.05.

3. Results and Discussions

The discovery of new, effective medications is closely related to phytochemical research. A basic step of qualitatively identifying the primary chemical constituent groups contained in the spices is made possible by the phytochemical screening research. Using color-forming and precipitating chemical reagents, phytochemical screening was performed on the extracts to get preliminary information on the components of the dried extract. The ethanolic extract was subjected to phytochemical screening using clove, garlic, black pepper, and bay leaf to investigate the existence of various therapeutic values such as proteins, glycosides, carbohydrates, steroids, alkaloids, flavonoids, and saponins. After their tests, four samples were selected for phytochemical analysis.

The current study discovered the presence of active components with possible health advantages after looking at four spice extracts: clove, garlic, black pepper, and bay leaf. The findings of a qualitative examination of the phytochemical active ingredients in each of these spices are shown in (Table 1). The antibacterial activity seen in the majority of the chosen spice extracts may be attributed to the presence of alkaloids, flavonoids, glycosides, phenols, saponins, steroids, and tannins. There were several spice extracts that were chosen but did not include alkaloids. Perhaps because of the presence of phytoconstituents such flavonoids, saponins, tannin, and phenols, the crude extract has antibacterial characteristics.

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Herbs with a high tannin content have an astringent quality [28, 29]. Sulaiman et al. reported that flavonoids and saponins were detected through phytochemical screening of several spice extracts, supporting their findings. These substances may help to explain the plant's many historic medicinal applications [23].

Table 1: Phytochemical screening of herbal extracts

Phytochemical					
Terpenoid					
Flavonoid					
Phlobatanin					
Tannins:					
Coumarin:					
Steroid:					
Saponin:					

Antibacterial activity of extracts:

Due to naturally existing combinations of phytochemical components, crude extracts exhibit a moderately reduced inhibitory efficacy compared to the standard medication against the tested bacteria and fungus. The extract's presence of components like flavonoids and tannins is probably what causes the antibacterial activity. The findings of this investigation indicated that each of the four spice extracts contains a number of phytochemicals and has antioxidant effects. These phytochemicals' existence can be explained by the fact that solvent extracts of some bacterial strains exhibited antibacterial action. The antibacterial efficacy of the spice extracts of Clove, Garlic, Black pepper, Bay leaf against the Gram-positive bacterial pathogen *Staphylococcus aureus* was investigated in this work. As per usual practices, spice extracts were made, filtered, and evaporated.

Individual extracts' antibacterial efficacy against the chosen pathogens was evaluated based on the presence or absence of an inhibition zone. Agar well diffusion assay was used to determine the zone of inhibition, and the diameters of the zones are displayed in (Table 2). Four medicinal plants' ethanol extracts were evaluated against virulent strains of *Staphylococcus aureus*, one of the most prevalent bacteria, as well as a pathogen that can infect wounds and cause septicemia, endocarditis, and toxic shock syndrome. Four spice species demonstrated antibacterial activity by suppressing microorganisms out of nine spice extracts that were tested for antimicrobial activity.

The results of the disc diffusion method used to test plant extracts' antibacterial activity against microbes are displayed in (Table 2) above. Clove (12 mm), Garlic (15 mm), Black pepper (19 mm), Bay leaf (17 mm) all have activity zones of inhibition against *staphylococcus aureus* bacteria. Numerous medicinal plants' antibacterial traits have been researched in the past. The antibacterial activity of spice extracts from nine significant medicinal plants was assessed in the current investigation against the bacterium *Staphylococcus aureus*. Positive controls were employed for the comparison. The test organisms were not inhibited by positive controls, in any way. The results are consistent with those of other researchers including Baljeet (2015) who investigation of the antibacterial effects of garlic on bacteria [25]. The ethanolic extract of Clove, Garlic, Black pepper, Bay leaf showed the greatest antibacterial action against gramme positive (*Staphylococcus aureus*) bacteria in the current trials among all the liquid extracts examined. While the results of this study and those of other researchers are in agreement, the diameter of the inhibition zone generated differs from those of other studies. The source of the microorganisms that were used may be the cause of the

discrepancy. Furthermore, both internal and extrinsic factors can influence a plant extract's ability to combat a specific disease (Figure 1).

Terpenoid:	1gm extract was mixed with 2ml chloroform and concentrated H_2SO_4.
Flavonoid:	1ml extract was mixed with NaOH and HCl.
Phlobatanin:	1 millilitre of extraction was mixed in purified water & processed. In a 2 percent HCl, the filtrate was diluted.
Tannins:	1% lead acetate was added in 1ml extract.
Coumarin:	In 2ml of extraction, 3ml of 10% NaOH was applied
Steroid:	1 mL extraction was mixed in 10 mL chloroform, and an appropriate amount of sulphuric Acid was applied to the test tube's sides.
Saponin:	One millilitre of extraction was combined with three millilitres of dw and allowed for shaken vigorously.

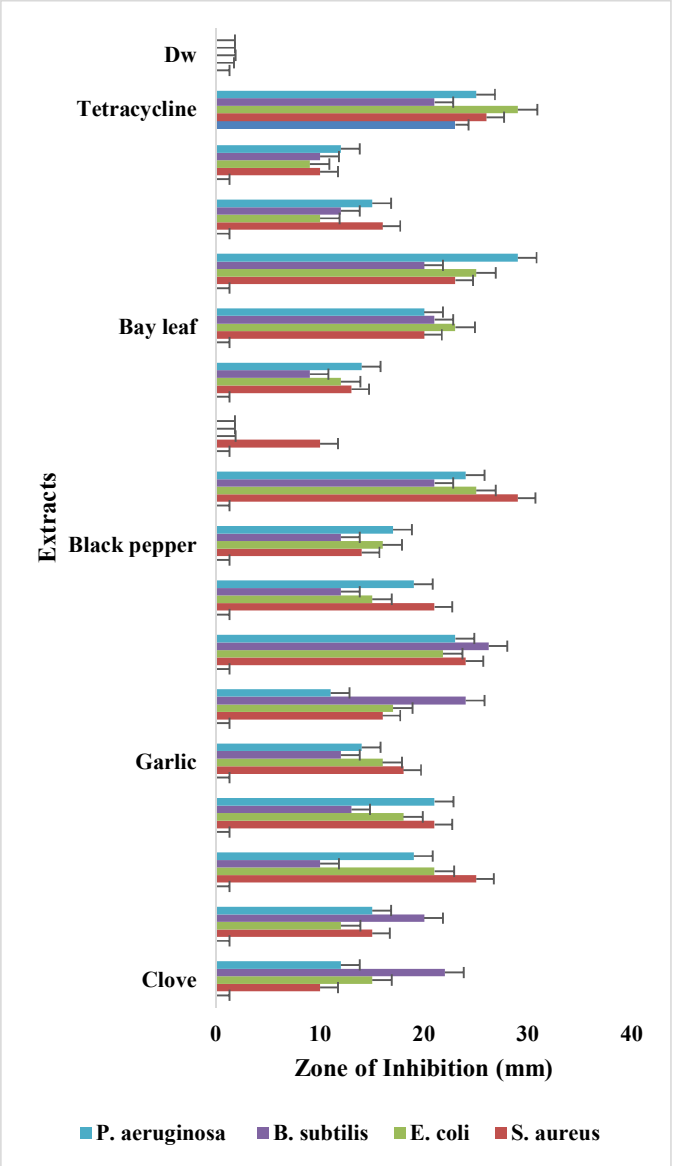

Figure 1: Antibacterial properties of extracts against gram positive and gram negative bacteria.

4. Conclusion

The current study reveals that the chosen spices include natural antioxidants and phytochemicals, which may be the reason for their usage in traditional medicine and in the treatment of infections and some disorders. It is advisable to promote the use of these spices or their extracts in the creation and development of functional foods. The findings of this study also offer convincing proof that most spices are currently underutilised as flavour or medicinal herbs. Based on these results, the study recommends emphasising the usage of spices while also raising public knowledge of their health advantages.

According to the results of the study mentioned above, the phytochemical components found in the ethanol crude extract of spices may be responsible for their antibacterial properties. The tested bacteria as well as Staphylococcus aureus were both susceptible to the crude extract. These results imply that the chosen phytoextracts have antibacterial characteristics and may be employed for bacterial culture biocontrol. The results showed that plant extracts varied in their effectiveness for preventing the bacterial development of the investigated diseases and that antimicrobial activity was confirmed by the chosen spice species.

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