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ORIGINAL RESEARCH ARTICLE

Phytochemical Analysis and Comparative Effect of *Cinnamomum zeylanicum, Piper nigrum* and *Pimpinella anisum* with Selected Antibiotics and Its Antibacterial Activity against Enterobacteriaceae Family

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ABSTRACT

In this present study, the spices *viz.*, *Cinnamomum zeylanicum, Piper nigrum,* and *Pimpinella anisum* were tested for its antibacterial activity against Enterobacteriaceae family. The Spices were tested against the organisms *such as Escherichia coli*, *Salmonella species, Shigella* sp., *Klebsiella* sp., and *Proteus* sp. The phytochemical components present in the spices were also assessed. The dried powder of *Cinnamomum zeylanicum, Piper nigrum, Pimpinella anisum* were extracted using organic solvents such as ethanol, methanol and chloroform. The extracts were tested for the presence of flavanoids alkaloids, cardiac glycosides, saponins, tannins, and terpenoids. Results showed that the phytochemical screening and qualitative estimation of the crude yield of *Cinnamonmum zeylanicum, Piper nigrum, and Pimpinella anisum* were rich in alkaloids, flavanoids, terpenoids, and saponins. The effect of *Cinnamomum zeylanicum, Piper nigrum and Pimpinella anisum* against the Enterobacteriaceae family was compared with the antibiotics *viz.*, Penicillin, Streptomycin, and Gentamycin.

Key words: Spices, Phytochemicals, Enterobacteriaceae and Antibiotics.

1. INTRODUCTION

A Spice is defined as natural compound that is extracted from the seeds, fruits, flowers or trunks (skin, roots, leaves) of several plants are add to food in order to provide taste smell or flavor. Spices are a diverse group of a wide variety of staple dietary additives consumed all over the world, significantly more tropical oriental, Hispanic and Mediterranean cuisines. The Spice is a culinary term not a botanical category it does not refer to a specific kind of plant or plant part [1]. Each spice has a unique aroma and flavor which derive from compounds known as Phytochemicals or secondary compounds. These chemicals evolved in plants to protect them against herbivorous insect vertebrates, fungi pathogens and parasites [2]. For centuries the inherent value as well as potential; toxicity of phytochemicals to human health has been recognized [3]. Spices are used as the substances that increase the taste and variation of food [4].

Spices are the important part of human diet; humans have been used spices from the thousands of years to enhance flavor, colour and aroma of food. Spices are used from the ancient time by physicians and laymen to treat a great variety of human resources. More recently, the interest in herbs and spices has grown not only for their seasoning andflavouring properties, but also for their antioxidant potential. In addition, such property has also demonstrated its importance in the prevention of some diseases. Consumption of herbs and spices has been implicated in the prevention of cardiovascular diseases, carcinogenesis, inflam mation, atherosclerosis, etc. [5, 6].

2. MATERIALS AND METHODS

The well known and commonly used Indian spices namely *Cinnamomum zeylanicum, Piper nigrum* and *Pimpinella anisum* were tested for its activity against Enterobacteriaceae family. Three different spices were purchased from the local market of Chidambaram.

Microorganism used

The clinical strains of bacterial species used were *Escherichia coli, Salmonella species, Klebsiella* sp., *Shigella* sp., *Proteus* sp. were isolated and identified and the biochemical and morphological characteristics were confirmed by standard method [7] organisms were sub cultured and maintained under the laboratory conditions.

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Preparation of extracts

For the preparation of extracts the method reported by Harborne [8] was used. Dried powder of the spices (10gm) was extracted with 50 ml of ethanol, methanol, chloroform. The mixture was left for 24 hours at room temperature. The extracts were concentrated to remove the solvent and filtered through whatmann No.3 filter paper.

Phytochemical screening of Extracts

The standard method of Harbarne [9] were used to test for the presence of Phytochemical in the test samples

Test for Alkaloids

2 ml of each extract was treated with 2 ml of Wagner's reagent. A brownish red precipitate indicates the presence of alkaloids.

Test for Cardiac Glycosides

2 ml of each extract was dissolved with 2 ml of chloroform and concentrated sulphuric Acid was carefully added to form a layer. Deep reddish brown color at the interface of steroid ring indicates the presence of Cardiac Glycosides.

Test for Flavanoids

2 ml of each extract was treated with 2 ml of 10% lead acetate yellowish green color indicates the presence of Flavanoids.

Test for Saponins

2 ml of each extract was dissolved with 2 ml of Benedict's reagent. Blue black precipitate indicates the presence of Saponins.

Test for Tannins

2 ml of each extract was treated with 0.1% of ferric chloride. Brownish green indicates the presence of Tannins.

Test for Terpenoids.

2 ml of each extract was mixed with 2 ml of chloroform and concentrated sulphuric acid was carefully added to form a layer. A reddish brown indicates the presence of Terpenoids.

Antimicrobial activity test using agar well method

In modified antibacterial test was performed using the agar diffusion method of Collins *et al*. [10]. The microorganisms were inoculated on Muller Hinton Agar and spread uniformly using sterile spreader. Wells of 5mm in diameter were made on Muller Hinton Agar using a sterile well puncher. The cut agar blocks were carefully removed by the forceps and sterilized by flaming. The crude extracts of the Spices were added to the well and control plates were maintained. The plates were allowed to stand for one hour at room temperature for diffusion of the substances before the growth of organism commenced. The plates were incubated at 37°c for 24 hours and then the zone of inhibition was recorded.

Antibiotic sensitivity testing

The test microorganisms were also tested for their sensitivity against the antibiotics of Gentamycin 10 mcg, Streptomycin 10 mcg, penicillin 10 mcg by the disc diffusion method. The cultures were enriched in sterile nutrient broth for 24 hours at 37c using a sterile cotton swabs. The cultures were aseptically swabbed on the surface of Muller Hinton Agar plates. Using an ethanol dipped and flamed forceps, the antibiotic disc were aseptically placed over the seeded Muller Hinton Agar plates sufficiently separated from each other to avoid overlapping of the inhibition zones. The plated were incubated at 37 °c for 24 hours and the diameter of the inhibition zones was measured in mm [11].

3. RESULTS

The extracts were separated from the spices using the solvents *viz*., ethanol, methanol and chloroform. The extracts of spices were tested for phytochemical analysis and its effect was tested for its antibacterial activity. The effects of Spices were also compared with the antibiotics and the results were as follows.

Phytochemical screening:

In the present study the spices samples showed the presence of phytochemicals. The presence of Phytochemical components such as tannins, alkaloids, terpenoids, cardiac glycosides, and saponins of the spices C*innamomum zeylanicum, Piper nigrum, Pimpenella anisum* were tabulated in (**Table 1, 2 & 3**). The presence of alkaloids was confirmed by producing reddish orange precipitate. The presence of tannins was confirmed by producing brownish green color. The presence of terpenoids was confirmed by producing reddish brown color of the interface. The presence of cardiac glycosides were confirmed by producing deep reddish brown color at interface a steroid ring present.

The flavanoids compound are absent in cinnamon extract at the same time the compound of tannins were absent in aniseed extract. The absence of yellowish green confirms the absence of flavanoids in cinnamon extract and the absence of blue black precipitate conforms the absence of saponins is aniseed extract. The organic solvents such as ethanol, methanol and chloroform were used These extracts were tested against Enterobacteriaceae family such as *Escherichia coli*, *Salmonella* species *Shigella* species *Proteus* spices, *Klebsiella* species.

The ethanol extracts of *Cinnamomum zeylanicum* shows sensitivity to *Escherichia* coli and *Proteus* species (**Table 4**). Methanol extract of cinnamon shows sensitivity to *Proteus* species Chloroform extract of cinnamon shows sensitivity to salmonella species and *Proteus* species. The ethanol extract of *Piper nigrum* shows sensitivity to *Klebsiella* species, *Shigella* spices, *Salmonella* species and *Proteus* species (**Table 5**). The methanol extracts of *Piper nigrum* shows sensitivity to *Salmomella, Proteus* species and shows resistance to *Escherichia* species *Klebsiella*, *Shigella* species. The chloroform extract of *Piper nigrum* shows sensitivity to *Proteus* species. (**Table 6**) showed the antibacterial activity of *Pimpenella anisum* the ethanol extract of *Pimpenella anisum* shows sensitivity to *Shigella* species and *Salmonella* species whereas, the ethanol, methanol, and chloroform extract of *Pimpenella anisum* shows resistance to *Escherichia* species, *Proteus* species and *Klebsiella* species.

Antibiotic sensitivity test:

The effect of antibiotics against Enterobacteriaceae family were tabulated in (**Table 7**) *Escherichia coli, Shigella* species, *Salmonella* species, *Proteus* species were sensitivity to Gentamycin, whereas *Escherichia coli, Salmonella* species, *Shigella* species, *Proteus* species *Klebsiella* species were resistance to penicillin and streptomycin antibiotics.

4. DISCUSSION

The Phytochemical screening and qualitative estimation of the crude yield of chemical constituent of the spices showed that the bark of cinnamon, fruit of pepper, seed of aniseed were rich in the alkaloids, terpenoids, tannins, Cardiac glycosides, flavanoids, saponins [12]. The qualitative analysis of Cinnamon, showed the presence of Phytochemical constituent alkaloids in ethanol, methanol extract cardiac glycosides, tannins, terpenoids and saponins are present, at the same time flavanoids are absent in the cinnamon [13].

The qualitative analysis from the fruit of *piper nigrum* showed the presence of Phytochemical constituent such as cardiac glycosides, tannins, terpenoids, saponins except chloroform extract, alkaloids, flavanoids, terpenoids were present [14].

The qualitative analysis from the seed of *Pimpinella anisum* showed the presence of Phytochemical constituent such as alkaloids, flavanoids, cardiac glycosides terpenoids, saponins except the tannins all the five compounds were present in aniseed [15].

Most of the strains used in this study were resistant to antibiotic. The increase of the microbial resistance to antibiotics is now being more and more of concern throughout the world. New antibiotics are being processed for alleviating this situation and some research works are also being carried out on natural compounds for achieving the destruction of these microorganisms [16]. Thus, it was evaluated that rather than use of streptomycin, penicillin antibiotics *Cinnamomum zeylanicum* and *Piper nigrum* extracts can be used as the antibacterial agent [17].

Table 1: Phytochemical screening of *Cinnamomum zeylanicum*

Phytochemical	Ethanol	Methanol	Chloroform
Alkaloids	++	++	+
Cardiac glycosides	+	+	++
Flavanoids	-	-	-
Tannins	++	++	++
Terpenoids	+	++	++
Saponins	++	+	+

Table 2: Phytochemical screening of *Piper nigrum*

Phytochemicals	Ethanol	Methanol	Chloroform
Alkaloids	+	++	-
Cardiac glycosides	+	++	+
Flavanoids	+	+	-
Tannins	++	++	++
Terpenoids	+	+	-
Saponins	++	++	++

Table 3: Phytochemical screening of *Pimpinella anisum*

Phytochemical	Ethanol	Methanol	Chloroform
Alkaloids	+	++	++
Cardiac glycosides	+	++	++
Flavanoids	++	++	+
Tannins	++	++	-
Terpenoids	+	+	+
Saponins	+	+	+

+ Positive; - negative; ++ deeply positive

Table 4: Antibacterial activity of *Cinnamomum zeylanicum*

Enterobacteriaceae Family	Ethanol	Methanol	Chloroform
Escherichia coli	15mm	13mm	11mm
Klebsiella species	14mm	No zone	11mm
Shigella species	14mm	13mm	Less than 10
Salmonella species	13mm	11mm	15mm
Proteus species	19mm	27mm	21mm

Table 5: Antibacterial activity of *Piper nigrum*

Enterobacteriaceae Family	Ethanol	Methanol	Chloroform
Escherichia coli	11mm	No zone	No zone
Klebsiella species	No zone	No zone	No zone
Shigella species	15mm	No zone	No zone
Salmonella species	14mm	15mm	No zone
Proteus species	11mm	13mm	11mm

Table 6: Antibacterial activity of *Pimpinella anisum*

Enterobacteriaceae Family	Ethanol	Methanol	Chloroform
Escherichia coli	11mm		
Klebsiella species	No zone	No zone	No zone
Shigella species	15mm	No zone	No zone
Salmonella species	14mm	15mm	No zone
Proteus species	11mm	13mm	11mm

Table 7: Antibiotic sensitivity test

Enterobacteriaceae Family	Antibiotics		
	Penicillin	Streptomycin	Gentamycin
Escherichia coli	No zone	No zone	16mm
Klebsiella species	No zone	No zone	14mm
Shigella species	No zone	13mm	16mm
Salmonella species	No zone	12mm	17mm
Proteus species	No zone	12mm	17mm

5. CONCLUSION

The present study deals with the Phytochemical screening and antibacterial activity of three spices *Cinnamomum zeylanicum*, *Piper nigrum* and *Pimpinella anisum*. The presence of phytochemicals in spices has the bacteriostatic and bactericidal activity. The antimicrobial substances contained in the extracts included tannins, alkaloids, terpenoids, flavanoids, cardiac glycosides and saponins It therefore suggests that constituents of spices extracts could serve as a source of drugs useful in the chemotherapy of diseases caused by the Enterobacteriaceae family. While comparing with the antibiotics spices showed good results hence, it can be used as the natural rather than use of antibiotics.

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