APPRAISAL OF NUTRITIONAL STATUS AND ANTIMICROBIAL ACTIVITY OF CLOVE, KALONJI, CINNAMON, BLACK PEPPER AND SWEET BASIL

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Summary

The aim of the present study was to assess the antimicrobial activities of crude ethanolic extracts of Clove (*Eugenia caryophyllus*), kalonji (*Nigella sativa* L.), Cinnamon (*Cinnamomum zylancium*) Black pepper (*Piper nigrum* L.) and Sweet Basil (*Ocimum bascilicum* L.) against selected Gram +ve and Gram -ve bacteria and also to evaluate the nutritional status of these culinary and medicinally important spices.Ethanolic crude extract of clove exhibited excellent antimicrobial activity against all tested microorganisms whereas Kalonji and Cinnamon extracts showed antibacterial activity against only Gram +ve bacteria. All tested microorganisms were resistant to Black pepper and Sweet Basil extracts. Analyzed spices were conforming the nutritional standards as reported in literature.

Key words: Spices extracts, Antibacterial activity, proximate analysis, clove, *Nigella sativa*.

Introduction

Plants have been a valuable source of natural products for a long period of time to maintain human health, especially with more intensive studies in the last decade for natural therapies. [1] Spices and herbs have been used for thousands of centuries by many cultures to enhance not only flavor and aroma of the foods but also to provide antimicrobial properties. Early civilizations recognized the value of using spices and herbs for their medicinal significances and foods preservation. [2, 3, 4] Spices can be defined as any dried, fragrant, aromatic or pungent vegetables or plant substances in whole, broken or ground forms that contribute flavor, whose primary function in food is seasoning rather than nutrition and that may contribute piquancy of foods and beverages.[5] In addition to these spices are some of the most commonly used natural antimicrobial agents in foods. Some of the natural compounds found in various spices possess antimicrobial activity. [6]

Since the introduction of antibiotics there has been tremendous increase in the resistance of diverse bacterial pathogens. This shift in susceptibility greatly affects ability to successfully treat patients empirically. [7] Therefore, actions must be taken to control this problem by using the plant extracts containing phytochemicals having antimicrobial properties.[1] In generally spices are considered safe and proved to be effective against certain ailments.[8] Various spices show varied levels of antimicrobial activity against diverse microorganisms depending upon their composition.[9]

Keeping in view this fact the present study was conducted to find out the antimicrobial activity of five spices including Clove (*Eugenia caryophyllus*, family Mytraceae), kalonji (*Nigella sativa* L.,family Ranunculaceae), Cinnamon(*Cinnamomum zylancium*, family Lauraceae) Black pepper (*Piper nigrum* L. family Piperaceae) and Sweet Basil (*Ocimum bascilicum* L. family Lamiaceae) against pathogenic bacteria. Bioactive components of these spices as reported [5, 6, 9, 10,11] in literature are presented in table-1.

Table 1: List of botanic features of analyzed spices and their antimicrobial components.

Spices	Botanical names of the plant	Name of plant part used as spice	Antimicrobial compounds
clove	*Eugenia caryophyllus*	Flower stalk, dried bud	Eugenol and eugenol acetate.
Kalonji	*Nigella sativa* L.	Seeds	Thymoquinone (TQ)
Cinnamon	*Cinnamomum zylancium*	Leaf, bark	Cinnamaldehyde and Eugenol .
Black pepper	*Piper nigrum* L	Fruit	Piperine
Sweet basil	*Ocimum bascilicum* L.	Leaf	linalol, estragol and eugenol

Clove (*Eugenia caryophyllus*) the aromatic dried flower buds are used in Ayurveda, Chinese medicine and Western herbalism. The principal active constituents of clove are biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanoic acid. [11] and the main constituents of clove essential oil are phenylpropanoides such as carvacrol, thymol, eugenol and cinnamaldehyde. Several studies have demonstrated potent antifungal, antiviral and antibacterial effects of clove. [12]

Kalonji (*Nigella sativa* L.) has been used for centuries by millions of people in Asia, Middle East and Africa. For thousands of years, human around the world have recognized the tremendous healing properties of this legendary herb. The black seed contain 36-38% fixed oil, alkaloids, saponins and fatty acids etc. Black seed extract or oil has been reported to possess antimicrobial, antioxidant and anti-tumor activity and a stimulatory effect on the immune system. The protective and healings powers are so comprehensive and varied that it is regarded as a miracle cure. [13, 14]

Cinnamon (*Cinnamomum zylancium*) has a long history both as a spice and as a medicine. Cinnamon oil contains active components called cinnamaldehyde, cinnamyl acetate, and cinnamyl alcohol, plus a wide range of other volatile substances. The activity of cinnamon is due to the presence of cinnamaldehyde (50.5%), which is an aromatic aldehyde that inhibits amino acid decarbooxylase activity and has been proven to be active against many pathogenic bacteria. [15]

Black pepper (*Piper nigrum* L.) is a flowering vine, native to India and has been a prized spice since ancient times. Similarly it has been shown to have antimicrobial activity. [16] Piperine a pungent alkaloid present in black pepper enhances the bioavailability of various structurally and therapeutically diverse drugs. It is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion, fever, intermittent fever, cold extremities, colic, gastric ailments and diarrhea. [17]

Sweet basil *(Ocimum bascilicum* L.) is native plant of Indo-Malayan region. It is called the "king of herbs" which contains plenty of phytochemicals with significant nutritional as well as antioxidant capabilities and health benefits. [10] Sweet Basil has shown unique health protecting effects due to its important flavonoids and volatile oils. The unique array of active constituents called flavonoids found in basil provides protection at cellular level**.** [18] Essentials oil obtained from basil leaves, has demonstrated the ability to inhibit several species of pathogenic bacteria that have become resistant to commonly used antibiotic drugs. [19]

Keeping in sight of their beneficial aspects of spices present study was conducted to evaluate their nutritional status and to asses their antimicrobial activity.

Materials and Methods

Selection of spices

Four medicinal spices; Clove (*Eugenia caryophyllus*), Kalonji (*Nigella sativa* L.), Cinnamon (*Cinnamomum zylancium*,) and black pepper (*Piper nigrum* L.) were collected from local market where as Sweet Basil leaves (*Ocimum bascilicum* L.) were harvested from crop grown in the garden of PCSIR Laboratories Complex, Ferozpur Road, Lahore.
These spices have previously been reported to have antimicrobial activity against different microbes. [5]

Preparation of spices extracts

To prepare spices extracts absolute ethanol (99%) was used as solvent. Took 10g of each of spices, macerated in 40 ml of absolute ethanol individually and vigorously stirred with a sterile glass rod and kept overnight. Extracts were occasionally shaken during 24h and then filtered through Whatman No.1 filter paper [20] repeated the extraction twice and discarded the remaining material thereafter. The filtrate of each spice having specific color and aroma, were evaporated to dryness on a water bath at 100±2ºC.

The dried extracts were sterilized by placing them under UV light for 24 hours. Each of the alcoholic extracts was reconstituted by adding 2mL of 10%(w/v) aqueous dimethylsulfoxide (DMSO) with Tween-80 (0.5% v/v) which was sterilized by filtration through a 0.45 µm membrane filter and used for further study immediately.

Test organisms

In vitro antimicrobial studies were carried out on eight bacterial strains (*Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Escherichia coli* and *Enterobacter* specie) some of which were obtained from PCSIR laboratories complex, Lahore and other from pathological laboratory of a local hospital. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

Antibacterial Assay

Paper disc diffusion method reported by Bauer *etal.,*[27] was applied to test the antimicrobial activity of the extracts. Discs of Whatman No.1 filter paper having a diameter of 6 mm were placed in a sterile Petri dish wrapped in tinfoil and oven dried at 65ºC overnight for sterilization.

Normal strength nutrient agar medium (OXOID, England) was prepared for determination of antibacterial activity of the spices extracts through out the study. Growth media was always autoclaved at 121ºC under 15psi for 15 minutes. For antibacterial assay 24h old bacterial cultures at 37ºC were used. Cultures were diluted 10^{-1} in sterile ringer solution [21] containing approximately 10^6CFU/mL in each case. Twenty five micro-liters of these suspensions were inoculated over plates containing sterile nutrient agar medium using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates.

Sterile filter paper discs each impregnated with 30µL of different spice extract were placed on pre-inoculated culture media under aseptic conditions separately and incubated at 37ºC for 24h. The zone of inhibition in each case was measured as the diameter (in millimeters) of the clear zone around the discs. All experiments were performed in duplicate. Penicillin and Streptomycin were used as positive controls. Inhibitory effect of positive controls was tested for all microorganisms used in this study under the incubation conditions as mentioned above. The working solution of control antibiotics were prepared in appropriate amounts (0.01g/10mL) then 25µL of each antibiotic solution was dropped on paper discs and 10 % aqueous solution of dimethylsulfoxide (DMSO) was used as negative control during this study.

Proximate analysis

Proximate composition of spices i.e. moisture, ash, protein, and fat were carried out according to standard methods of A.O.A.C (1990). [22] All the tests were carried out in triplicate.

Results and Discussion

The data pertaining to the antimicrobial potential of the crude ethanolic extract of spices is presented in table-2. The crude ethanolic decoction of clove exhibited antimicrobial activity against all tested Gram +ve and Gram –ve microorganisms with an inhibition zone diameter (IZD) ranging from 16.25mm to 25.25mm as shown in table-2. The Inhibition zone diameter was maximum against *Bacillus subtilis* (25.25±0.33 mm), followed by *Enterobacter* species (23±2.12mm), *Staphylococcus aureus*(21±0.86mm), *Escherichia coli* (20.5±0.71mm), *Escherichia coli* ATCC 25922(19.75±0.36mm), *Klebsiella pneumoniae* (18±0.35mm) and *Salmonella typhimurium* (16.50±0.36mm).

Nazrul Islam *et al.,* [24] reported that clove ethanolic extract was effective against *Bacillus subtilis* with an IZD of 17mm which is contrary to the present findings where clove extract showed a higher IZD of 25.25±0.33 mm against *Bacillus subtilis*. In a study carried out by Betoni *et al.,* [23] Clove extract showed inhibitory effect against S. aureus. These findings are also in harmony to present findings. Nazrul Islam *et al.,* [24] accounted that *Pseudomonas aeruginosa* was less susceptible to ethanolic extract of clove with an IZD of 9mm which is in contradiction to our findings where *Pseudomonas aeruginosa* exhibited an IZD of 16.25± 0.35mm against ethanolic extract of clove. These results are supported by the findings of Sabahat *et al.,* [11] who reported that aqueous decoction of clove exhibited maximum activity against *P. aeruginosa* with an IZD of 10.86± 1.46mm among all tested microorganisms.

Furthermore, present results depicted that antimicrobial activity of clove extract against tested microorganisms were comparable to the positive control i.e. Streptomycin whereas clove extract was found to be more active against *Klebsiella pneumoniae* (IZD of 18±0.35mm) as compared to Streptomycin (IZD of 16.75±0.36mm) as shown in table-2. Similarly, Nazrul Islam *et al.,* [24] found that etahanolic clove extract inhibited growth of Klebsiella sp. with inhibition zone diameter of 15mm.

In the present research, the antibacterial activity of ethanolic crude extract of Kalonji was next to clove. It exhibited inhibitory action against only *Bacillus subtilis* and *S. aureus.* The inhibition zone diameter of *Bacillus subtilis* and *S.aureus* against Kalonji ethanolic decoction was 12.3±0.92 and 16±0.28 respectively. These results are correlated with the findings of Nazia *et al.,* [5] who reported that aqueous decoction of kalonji inhibited the growth of *Staphylococcus aureus* with an IZD of 19.6mm ± 1.8mm. All other tested G –ve bacteria were resistant to kalonji extract. In another study, *Salman et al.,* [25] reported that Gram +ve bacterial isolates were more sensitive to aqueous decoction of kalonji than Gram -ve microorganisms. The active ingredient of kalonji seeds was thymoquinone (TQ), which is responsible for antibacterial activity. [5, 13]

Table-2: Assessment of antimicrobial activity in four different extracts of *O. basilicum* against eight pathogenic microorganisms

Test Micro-organisms	**Antimicrobial activity of spices Zone of inhibition (mm)**							
	Clove 30 ul/disc	**Kalonji 30 ul/disc**	**Cinnamon 30 ul/disc**	**Black pepper 30 ul/disc**	**Sweet Basil 30 ul/disc**	**DMSO 30ul/disc**	**Streptomycin 25ug/disc**	**Penicillin 25ug/disc**
Escherichia coli ATCC 25922	19.75 ± 0.36	-	-	-	-	-	24.5± 1.41	19±0.12
Bacillus subtilis ATCC 6633	25.25 ± 0.33	12.3± 0.92	8.5± 0.21	-	-	-	25.50± 0.71	11.15± 0.30
Klebsiella pneumoniae HI[a]	18± 0.35	-	-	-	-	-	16.75± 0.36	-
Salmonella typhimurium HI	16.5± 0.36	-	-	-	-	-	22.5± 0.35	-
Pseudomonas aeruginosa HI	16.25 ± 0.35	-	-	-	-	-	21±1.06	-
Staphylococcus aureus HI	21± 0.86	16± 0.28	9.2± 0.28	-	-	-	21± 0.76	17± 1.73
Escherichia coli HI	20.5± 0.71	-	-	-	-	-	23±0.35	20±0.24
Enterobacter species HI	23± 2.12	-	-	-	-	-	20±0.35	-

[a] Hospital isolated pathogen
(-) No inhibition zone (resistant)

Present study depicted that the ethanolic crude decoction of Cinnamon was effective against only *S. aureus* and *B. subtilis* with an inhibition zone diameter of 9.2±0.28mm and8.5±0.21mm respectively. These findings are in agreement to Agaoglu *et al*., [9] who reported that most susceptible bacteria to cinnamon extract was *S. aureus*. Present findings showed that Cinnamon decoction was not effective against tested Gram –ve bacteria. Nanasombat *et al.*, [6] reported that was resistant to cinnamon extract which correlates with present findings. Agaoglu *et al.*, [9] also reported that cinnamon has inhibitory effect on *P.aeruginosa*, *E. faecalis* and *E. coli.* These results are in confliction to present findings where *P.aeruginosa*, Enterobacter species and *E.coli* were resistant to cinnamon extract.

Both spices Sweet basil and Black pepper showed no antimicrobial effect against tested strains. Regarding the results of Black pepper these findings are in accordance to Ghori et al. who reported that black pepper dissolved in DMSO showed no inhibition against *S. aureus*, Salmonella sp, *E. coli and B. subtillus.* However many other investigators reported about the antibacterial activity of black pepper. [5, 26] All the tested microbes were resistant to crude ethanolic extract of Sweet Basil. These findings correlated with Nanasombat *et al.*, [6] who stated that ethanolic extracts of holy basil, black pepper and white pepper were inactive against all bacterial strains tested.

Proximate analysis

The results of proximate composition of tested spices are presented in table-3 Among the analyzed selected spices the highest moisture contents were found in Black pepper (10.82±2.16) followed by Clove (9.67±1.20)> Sweet basil (8.11±0.95)>Cinnamon (7.48±2.26)>Kalonji (7.043±1.07). In case of Ash contents results in ascending order are Sweet basil (5.34±0.53) > Black pepper (4.91±1.40) > Clove (4.62±0.45) > Kalonji (3.93±0.45) > Cinnamon (3.11±1.72). Regarding Fat contents of tested spices, results are in following order Kalonji (13.98±4.38) > Clove (13.58±1.75) > Cinnamon (8.99±0.28) >Black pepper (8.88±1.43)> Sweet basil (4.45±0.33). Concerning the protein contents of analyzed spices, results are in following order Sweet basil> Kalonji > Black pepper > Clove > Cinnamon

Table-3: Proximate analysis of Clove, Kalonji, Cinnamon, Black pepper and Sweet Basil.

Sr.#	**Name of spices**	**Moisture%**	**Ash%**	**Fat %**	**Protein%**
1.	Clove	9.67±1.20	4.62±0.45	13.58±1.75	5.88±1.01
2.	Kalonji	7.043±1.07	3.93±0.45	13.98±4.38	11.55±0.77
3.	Cinnamon	7.48±2.26	3.11±1.72	8.99±0.28	2.91±0.37
4.	Black pepper	10.82±2.16	4.91±1.40	8.88±1.43	11.15±0.45
5.	Sweet basil	8.11±0.95	5.34±0.53	4.45±0.33	12.11±1.0

Conclusion

Present study reaveled that among the analyzed spices Clove, Kalonji and Cinnamon had showed the effective antimicrobial activity against pathogens than the traditional antibiotics. This scientific information can serve as an important platform to develop an economic and safe alternative to treat infectious diseases.

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