Nepal J Biotechnol. 2021 Jul; 9 (1): 1-7 Research article DOI: https://doi.org/10.3126/njb.v9i1.38644

Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal
Journal Homepage: www.nepjol.info/index.php/njb
ISSN (Online): 2467-9313
ISSN (Print): 2091-1130

Antibiogram and Phytochemical Analysis Of Cinnamon, Clove, and Sichuan Pepper Extracts.

Bibek Adhikari [1,2], Pradeep Kumar Shah[1], Roman Karki[2]

[1] Department of Microbiology, Tri-Chandra Multiple Campus, Ghantaghar, Kathmandu, Nepal

[2] National Food Research Centre (NFRC), Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur, Nepal

Received: 07 Aug 2020; Revised: 08 Apr 2021; Accepted: 16 Apr 2021; Published online: 31 Dec 2021

ABSTRACT

A wide range of medicinal plant extracts has phytochemicals that possess antimicrobial properties and these plants are used to treat several infections. The study aimed to assess the antimicrobial activities of some spices extracts and to evaluate the phytochemicals present in them. The extracts of spices were prepared using Soxhlet apparatus refluxing with methanol and ethanol. The well diffusion technique was implemented for the evaluation of antimicrobial activities of the extracts and the zone of inhibitions was recorded in millimeters. The antimicrobial test was done against five bacterial isolates: *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella enterica* serotype Typhi, and *Staphylococcus aureus* and a fungal isolate: *Candida albicans*. The extracts were concentrated by Rotary Vacuum Evaporator and a stock solution of 200 mg/mL was prepared by dissolving in 10 % DMSO. Concentrations of 40, 60, 80 and 100 mg/mL extracts were used for antimicrobial activity. The result of this study showed that clove extracts had the highest antimicrobial property against all the test microorganisms. Methanolic extract of clove had the highest inhibitory effect against *Proteus mirabilis* (24.21±0.15 mm), *Pseudomonas aeruginosa* (19.78±0.23 mm), and *Candida albicans* (20.07±0.08 mm) whereas ethanolic extract was effective against *Escherichia coli* (20.44±0.16 mm), *Salmonella* Typhi (21.66±0.31 mm) and *Candida albicans* (21.11±0.09 mm). Cinnamon and pepper extracts, leaving some exceptions, also had antimicrobial properties. The presence of phytochemicals: polyphenols, flavonoids, and tannins are the major components responsible for antimicrobial activity. Thereby, this study successfully demonstrated the possibilities of using spices extracts in the treatment of microbial infections.

Keywords: Antimicrobial Activity, DMSO, Ethanol, Methanol, Phytochemicals.

Corresponding Author, Email: a.bibek52@gmail.com

Introduction

Herbal medicine or phytomedicine is the use of plants for medicinal and therapeutic purposes for the curing of diseases and improving human health [1]. A large portion of the world population, especially in developing countries, relies on the traditional systems of medicine to treat a variety of diseases [2]. Presently, more than two-thirds of the world's population leans on plant-based medicines relying on the fact that they are harmless and efficient against various afflictions [3, 4].

Abundant molecules with antimicrobial properties are present in medicinal herbs. Several infectious diseases are treated using a wide range of plant extracts since they possess antimicrobial potentials. Noticing the side effects on human health due to synthetic drugs, professionals are on the way to getting advantages from medicinal plants. A wide variety of screened plant molecules are traded as raw materials for several herbal preparations in the market. Out of reported 422,127 worldwide plant species, approximately 35,000 to 70,000 plant genera are utilized for medicinal purposes [5, 6].

Plants have secondary metabolites called phytochemicals (Phyto from Greek - meaning plant) that protect plants against microbial infections or pests infestations. Phytochemicals are active ingredients that possess therapeutic properties that are considered as a medicine or drug [1]. Alkaloids, flavonoids, phenolic compounds, and tannins are the essential phytochemicals present in the plants with possible therapeutic activities and these phytochemicals obtained in plant extracts have demonstrated antimicrobial potential against a wide range of infectious microorganisms [7, 8].

Microbial infection is a prevailing health problem around the world. Plants remain one of the potential sources of effective agents against microbes, including the deadly infection like tuberculosis (*Mycobacterium tuberculosis*), syphilis (*Treponema pallidum*), gonorrhea (*Neisseria gonorrhoeae*), skin and wound infections [9], diarrhea [10], typhoid fever (*Salmonella* Typhi), and *Pseudomonas aeruginosa* which directly infects the urinary tract, the pulmonary tract, wounds, burns and also causes other blood infections [8].

Microbial infection has been a major cause of death globally. The rapid increase in the development of resistance to antimicrobial agents by the microorganisms has led to the new incidence and re-exposure of disease

making them difficult and expensive to treat. To overcome these problems Pharmaceutical industries are in search of alternative antimicrobial agents. Spices possessing a wide range of bioactive compounds such as alkaloids, polyphenols, flavonoids, tannins, saponins, and various antioxidants have great potential as antimicrobial agents that can counteract pathogenic microorganisms. The spices extract solely or in combination with other antibiotics have the potential to work effectively against several infectious microorganisms [9].

Cinnamomum verum (Syn. *C. zeylanicum*) also known as Cinnamon is a small evergreen tree belonging to the family Lauraceae. The volatile oil produced from its leaf and barks are used as a flavoring agent in the food and beverage industry [11,12]. It is also used to treat abdominal pain, impotence, frigidity, dyspnoea, inflammation of the eye, leukorrhoea, vaginitis, rheumatism, neuralgia, wounds, toothache, and diabetes [11]. The principal constituents of leaf, bark and root oils are eugenol, cinnamaldehyde, and camphor, respectively [12,13]. Eugenol has been reported to inhibit the growth of *Escherichia coli* O157:H7 and *Listeria monocytogenes* [14]. Cinnamaldehyde has been reported to inhibit the growth of *Staphylococcus aureus*, *E. coli* O157:H7, and *Salmonella typhimurium* [15,16].

Syzygium aromaticum also known as Clove is the aromatic dried flower buds of a perennial tree belonging to the family Myrtaceae. Essential oil of clove is used as anodyne for dental emergencies, and against acne, warts, scars, and parasites. Also, the clove has antimutagenic, anti-inflammatory, antioxidant, antiulcerogenic, antithrombotic, and antiparasitic, antibacterial, and anti-inflammatory properties [3]. Research has shown that clove oil is an effective mosquito repellent [17]. The major constituents of clove's essential oil are carvacrol, thymol, eugenol, and cinnamaldehyde. Several studies have demonstrated potent antifungal, antiviral and antibacterial effects of clove [18]. Aqueous clove infusion was found to inhibit the growth of germinated spores of *Bacillus subtilis*, and inhibit the pathogens *Campylobacter jejuni*, *Salmonella enteritidis,* and *Escherichia coli* [19]. Clove oil exhibited antibacterial activity against *S. epidermidis*, *Salmonella* Typhi, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus* [20].

Zanthoxylum armatum, commonly known as Sichuan Pepper, belongs to the Rutaceae family. The bark, fruits, and seeds of Pepper are extensively used in the indigenous system of medicine as a carminative, stomachic, and anthelmintic. The seed and bark are also used as an aromatic tonic in fever, dyspepsia. Because of their deodorant, disinfectant, and antiseptic properties, the fruits are used in dental troubles, their lotion for scabies, and also used to ward off houseflies. Besides this, it is also used as a flavoring agent in the confectionery industry, and the manufacturing of soft drinks [21]. Pepper consists of several phytochemicals such as chlorogenic acid, cinnamic acid, epicatechin, rutin, trifolin, quercitrin, etc. which have anthelmintic, antifungal, and anti-insecticidal activities [22,23]. Different solvent extracts of pepper demonstrated antimicrobial properties against several pathogenic such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* Typhi, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* [24].

Materials and Methods

Plant Samples Collection

Plant materials used in the study consisted *Cinnamomum verum* (Cinnamon), *Syzygium aromaticum* (Clove), and *Zanthoxylum armatum* (Sichuan Pepper) which were collected from different parts of Nepal (**Table 1**). Since these three spices are most commonly found in the Nepalese kitchen, the authors selected these spices for antimicrobial and phytochemical analyses.

Table 1. Nomenclature of the spices and the locality from which they were obtained for this study.

Common Name	Scientific Name	Part used	Collected from
Cinnamon	*Cinnamomum verum*	Bark	Kathmandu
Clove	*Syzygium aromaticum*	Bud (fruit)	Kathmandu
Sichuan Pepper	*Zanthoxylum armatum*	Fruit	Salyan

Chemicals

Barium chloride, Dimethyl Sulfoxide, Folin-Ciocalteu reagent, Folin-Denis reagent, Methanol, and Sodium chloride were purchased from Thermo Fisher Scientific India Pvt. Ltd. Similarly, ethanol was purchased from Jiangyin Tenghua Co. Ltd., China, and Gallic acid and Tannic acid were purchased from Loba Chemie Pvt. Ltd., India. Additionally, Gentamicin, Muller Hinton agar (MHA), Nutrient agar (NA), Potato Dextrose agar (PDA), and sterile swabs were purchased from HiMedia Laboratories Pvt. Ltd., India.

Microorganisms

The pure cultures of *Candida albicans, Esherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica* serotype Typhi, and *Staphylococcus aureus* isolated from patients were obtained from the Department of Microbiology, Tribhuvan University Teaching Hospital (TUTH),

Maharajgunj, and the culture of *Proteus mirabilis* were obtained from Nepal Academy of Science and Technology (NAST), Khumaltar. The bacterial samples were maintained on NA and fungal samples on PDA at 4°C for further experiments.

Gentamicin

10 µg disc of Gentamicin was used as a positive control against bacterial cultures.

Extract Preparation

Air-dried, 30 g spices powder was taken in 120 mL of absolute methanol and absolute ethanol for 48 hours in Soxhlet apparatus (Borosil, India) separately and then Whatman no. 1 filter paper was used to filter the extract and allowed to evaporate using Rotary Vacuum Evaporator (Buchi Type) (Victo Lab, India) at 65°C for methanolic extract and 50°C for ethanolic extract [7, 10]. The concentrated extracts were stored in small containers in a refrigerator (4°C) for further use.

Standard concentration preparation

Two grams of each alcoholic extract were taken in a vial and 1mL, 100% Dimethyl Sulphoxide (DMSO) was added and dissolved in 9 mL of sterile double distilled water. Thus, 200 mg/mL of stock was obtained as a standard concentration. The extracts were then autoclaved (Victo Lab, India) for 20 minutes at 121°C and 15 pounds per square inch pressure [23].

Inoculum preparation

A colony of the organism from the stored agar plate was taken, transferred to a test tube containing 2.5mL sterilized NB and incubated for about 4 hours at 37°C in the incubator (Sanjeev Scientific Udhyog, India) and standardized the turbidity to 0.5 McFarland solution, microbial load equivalent to $1.5x10^8$ CFU/mL [24].

Antimicrobial Assay by Well Diffusion Method

MHA plates were prepared and test organisms were inoculated by spreading the bacterial inoculum on the surface of the media with the help of a sterile swab. Wells of 6 mm diameter were punched in the agar by using a cork borer. 50 µL of extracts with the concentration of 40, 60, 80, and 100 mg/mL were poured into the well. 50µL of absolute methanol, ethanol, and DMSO (10%) were used as controls. The plates were incubated at 37°C for 24 hours. Measurement of the diameter of the inhibition zone was done to evaluate the antimicrobial property with the help of a Vernier Caliper and recorded in mm [25].

Phytochemical Analysis

The extracts of the spices were prepared as described by Dimitrijević *et al* (2014) [26] and some modifications made by Bhattarai *et al* (2019) [27]. 5 g powdered spice was ground with 80 % methanol (30 mL) and was kept in an orbital shaker (Accumax, India) shaking continuously for about 20 minutes, and filtered through Whatman No. 1 filter paper in a 100 mL volumetric flask. The residue was again subjected to two more extractions with 30 mL each of 80 % methanol for a total time frame of an hour. The volume was made up to 100 mL using 80% methanol and the extracts were stored in a refrigerator at 4°C until further use.

Polyphenol Content

The polyphenol content of sample extracts was measured by using the Folin-Ciocalteu method, as described by Mahdavi *et al* (2010) [28]. 1 mL of extract was decanted in a volumetric flask of 25 mL containing 9 mL of distilled water. 1 mL of Folin-Ciocalteu reagent was added and shaken. After 5 minutes, 10 mL of 7 % Na_2CO_3 solution was added and the volume was made up with distilled water and mixed. The absorbance was measured at a wavelength of 765 nm using a Uv-Vis spectrophotometer (Genesys, USA) against a prepared reagent blank (distilled water), after incubation for 90 min at room temperature. The polyphenol content was reported as mg gallic acid equivalent per 100g sample (mg GAE/100g). A calibration curve was created from the standard and used to determine the corresponding gallic acid concentration of the samples.

Flavonoid Content

Aluminum trichloride ($AlCl_3$) assay as described by Samatha *et al* (2012) [29] with some modification done by Faleye *et al* (2018) [30] was used for the determination of flavonoid content. 0.5 mL of 80 % methanol extract was taken in different test tubes followed by the addition of 2 mL of distilled water and 0.15 mL of sodium nitrite (5 % $NaNO_2$, w/v). After 6 minutes, 0.15 mL of aluminum trichloride (10 % $AlCl_3$) was added and incubated for 6 min, followed by the addition of 2 mL of sodium hydroxide (NaOH, 4 % w/v), and volume was made up to 5 mL with distilled water. After 15 min of incubation, absorbance at 510 nm was measured against a reagent blank of distilled water. The calibration standard curve was prepared by preparing gallic acid solutions and the result was expressed as mg of gallic acid equivalents per 100g (mg GAE/100g) of the sample.

Table 2. Antibiogram of Methanolic Extracts

Spices	Conc (mg/mL)	Zone of Inhibition (ZOI) mm					
		E. coli	*P. mirabilis*	*P. aeruginosa*	*S.* Typhi	*S. aureus*	*C. albicans*
Cinnamon	40	0	0	0	0	0	0
	60	0	0	0	9.02±0.08^{b}	0	7.16±0.07^{a}
	80	6.60±0.10^{a}	7.89±0.10^{a}	0	10.19±0.07^{c}	6.52±0.11^{a}	8.02±0.06^{b}
	100	7.20±0.11^{ab}	9.02±0.06^{b}	0	11.12±0.05^{d}	7.87±0.09^{b}	10.00±0.07^{c}
Clove	40	10.62±0.18^{d}	14.90±0.42^{d}	11.61±0.22^{d}	10.22±0.05^{c}	11.69±0.16^{c}	16.50±0.21^{d}
	60	14.53±0.23^{f}	18.63±0.30^{e}	13.99±0.14^{e}	11.60±0.26^{de}	13.60±0.23^{d}	18.48±0.12^{e}
	80	17.19±0.34^{g}	21.60±0.26^{f}	16.15±0.22^{f}	15.66±0.11^{g}	16.68±0.11^{e}	19.52±0.07^{f}
	100	18.43±0.19^{h}	24.21±0.15^{g}	19.78±0.23^{g}	18.95±0.26^{h}	17.41±0.16^{f}	20.07±0.08^{g}
Pepper	40	7.54±0.14^{b}	7.99±0.10^{a}	0	9.35±0.15^{b}	10.99±0.14^{c}	0
	60	8.73±0.20^{c}	12.36±0.21^{c}	7.07±0.20^{a}	12.06±0.22^{e}	11.43±0.21^{c}	0
	80	12.97±0.08^{e}	14.4±0.12^{d}	8.46±0.16^{b}	13.46±0.06^{f}	16.65±0.18^{e}	0
	100	15.29±0.24^{f}	15.06±0.09^{d}	9.74±0.10^{c}	15.12±0.21^{g}	16.99±0.08^{ef}	0
Controls	Absolute Methanol	7.15±0.02^{bc}	7.66±0.24^{a}	7.14±0.27^{a}	7.24±0.07^{a}	7.19±0.14^{ab}	7.45±0.13^{a}
	Gentamicin	20.83±0.02^{i}	18.70±0.08^{e}	29.50±0.03^{h}	36.63±0.11^{i}	21.84±0.08^{g}	ND

ND= Not Determined

The measurements are the mean values (n, 3) ± standard error of the mean, whereas different alphabets vertically indicate a significant difference at 0.05 probability level.

Table 3. Antibiogram of Ethanolic Extracts

Spices	Conc (mg/mL)	Zone of Inhibition (ZOI) mm					
		E. coli	*P. mirabilis*	*P. aeruginosa*	*S.* Typhi	*S. aureus*	*C. albicans*
Cinnamon	40	7.60±0.11^{abc}	0	0	0	0	7.42±0.03^{a}
	60	7.94±0.50^{bc}	8.99±0.10^{a}	0	0	0	9.11±0.45^{b}
	80	8.12±0.08^{c}	10.06±0.05^{abc}	0	9.57±0.30^{a}	7.78±0.08^{bc}	11.14±0.36^{c}
	100	10.28±0.08^{d}	11.97±0.09^{de}	0	14.41±0.30^{b}	8.14±0.15^{c}	12.59±0.18^{d}
Clove	40	10.25±0.12^{d}	10.41±0.06^{bc}	6.97±0.13^{a}	19.13±0.08^{c}	12.5±0.06^{d}	17.64±0.48^{e}
	60	13.12±0.19^{e}	11.15±0.37^{cd}	9.20±0.10^{b}	19.51±0.18^{cd}	14.42±0.16^{e}	18.75±0.39^{ef}
	80	15.45±0.29^{f}	12.38±0.68^{e}	10.84±0.17^{c}	20.25±0.12^{d}	15.10±0.11^{f}	19.28±0.35^{f}
	100	20.44±0.16^{g}	13.90±0.05^{f}	11.78±0.08^{d}	21.66±0.31^{e}	15.72±0.08^{f}	21.11±0.09^{g}
Pepper	40	0	0	0	0	0	0
	60	0	0	0	0	0	0
	80	6.75±0.02^{a}	0	0	0	7.48±0.12^{ab}	0
	100	7.08±0.07^{ab}	0	0	0	8.02±0.12^{c}	0
Controls	Absolute Ethanol	8.19±0.26^{c}	9.98±0.09^{ab}	6.75±0.13^{a}	8.87±0.27^{a}	7.06±0.01^{a}	7.28±0.14^{a}
	Gentamicin	20.83±0.02^{g}	18.70±0.08^{g}	29.50±0.03^{e}	36.63±0.11^{f}	21.84±0.08^{h}	ND

ND= Not Determined

The measurements are the mean values (n, 3) ± standard error of the mean, whereas different alphabets vertically indicate a significant difference at 0.05 probability level.

Tannin Content

The Folin-Denis method was used for the determination of tannin content [31]. In a test tube containing 7.5 mL of distilled water, 0.1 mL of the sample extract was added. 0.5 mL of Folin-Denis phenol reagent and 1 mL of 0.5 % Na_2CO_3 solution were also added and diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. Absorbance at 775 nm was measured against the reagent blank and a set of reference standard solutions of gallic acid. The tannin content was expressed in terms of mg tannic acid equivalent per 100g (mg TAE/100g) of extract.

Statistical Analysis

Experimental analyses were performed in triplicates. IBM SPSS (Statistical Package for Social Sciences) Statistics version 20 was used for the statistical analysis. One-way Analysis of variance (ANOVA) was analyzed and the significant differences among them were studied by using Tukey HSD at a 5 % level of significance.

Results

Antimicrobial Activity by Well Diffusion Method

Agar well-diffusion method was employed to evaluate the antibacterial and antifungal activities of different concentrations (40, 60, 80, and 100 mg/mL) of the spice extracts. The result obtained by the well diffusion method has been summarized in **Table 2** and **Table 3**. The zone of inhibition greater than 6.5 mm has been taken into account. From **Table 2 t**he results obtained in this study revealed that the bacterial strains: *E. coli*, *P. mirabilis*, *P. aeruginosa*, *S.* Typhi, and *S. aureus*, and a fungal strain: *C. albicans* to be susceptible against the methanolic and ethanolic extracts of the three distinct spices. Both the methanolic and ethanolic extracts of Cinnamon had a

similar effect against test microorganisms, with little to no effect at lower concentrations and distinctive effects at higher doses. However, *P. aeruginosa* was found to be resistant to both extracts of Cinnamon. At 100 mg/ml concentration, the ZOI measurement of methanolic extract of Cinnamon against *E. coli*, *P. mirabilis*, *S.* Typhi, *S. aureus*, and *C. albicans* were 7.20±0.11, 9.02±0.06, 11.12±0.05, 7.87±0.09, and 10.00±0.07 mm; whereas 10.28±0.08, 11.97±0.09, 14.41±0.30, 8.14±0.15, and 12.59±0.18 mm by the ethanolic extracts of the same concentration.

The methanolic extract of Clove demonstrated higher antimicrobial activity against *P. mirabilis*, *P. aeruginosa*, and *S. aureus* with the ZOI measurement of 24.21±0.15 mm, 19.78±0.23 mm, and 17.41±0.16 mm at 100 mg/ml. Whereas ethanolic extract was comparatively found to be most effective against *E. coli*, *S.* Typhi, and *C. albicans* with 20.44±0.16 mm, 21.66±0.31 mm, and 21.11±0.09 mm inhibition zone at the same concentration.

The methanolic extract of Pepper was effective against all the bacterial strains: *E. coli*, *P. mirabilis*, *P. aeruginosa*, *S.* Typhi, and *S. aureus* with the ZOI of 15.29±0.24 mm, 15.06±0.09 mm, 9.74±0.10 mm, 15.12±0.21 mm, and 16.99±0.08 mm at 100 mg/ml and had no any effect against fungal strain i.e., *C. albicans*. However, the ethanolic extract had a selective activity against *E. coli*, and *S. aureus* with the ZOI measurement of 7.08±0.07 mm, and 8.02±0.12 mm respectively at the highest concentration i.e., 100 mg/ml.

Also, absolute methanol and absolute ethanol used as controls demonstrated antimicrobial property to some extent. However, 10% DMSO (data not shown) had no significant actions against the tested microorganisms.

Table 4. Phytochemicals present in Spices

Spices	Polyphenol (mg GAE/100g)	Flavonoid (mgGAE/100g)	Tannin (mg TAE/100g)
Cinnamon	362.95±19.49^{b}	329.29±1.90^{b}	326.86±7.96^{b}
Clove	455.86±9.91^{c}	399.70±5.34^{c}	402.24±4.25^{c}
Sichuan pepper	188.48±7.65^{a}	117.52±2.68^{a}	64.82±1.89^{a}

GAE= Gallic Acid Equivalent, TAE= Tannic Acid Equivalent

The measurements are the mean values (n, 3) ± standard error of the mean, whereas different alphabets vertically indicate a significant difference at 0.05 probability level.

Phytochemical properties

The spices used for the experiment demonstrated a decent amount of phytochemical properties which have been tabulated in **Table 4**. Among all the spices used, clove had the highest amount of phytochemical properties and pepper showed the lowest. The polyphenol content in spices ranged from 188.48±7.65 to 455.86±9.91 mg GAE/100g (y = 0.0178x, R^2 = 0.9356; where x, y, and R^2 represent concentration, absorbance, and correlation coefficient respectively). The flavonoids varied from 117.52±2.68 to 399.70±5.34 mg GAE/100g (y = 0.0088x, R^2 = 0.9924) whereas the range of tannin contents were found to be in between 64.82±1.89 to 326.86±7.96 mg TAE/100g (y = 0.0022x + 0.0055, R^2 = 0.9767). The phytochemicals followed a similar trend: Polyphenol> Flavonoids> Tannins, only the exception being Clove where Tannin contents were slightly higher than the flavonoid content.

Discussions

Dose-reliant antimicrobial activity of extracts was observed, which means with the increase in extract's concentration (from 40 to 100 mg/mL) the lethal effect was more pronounced.

The methanolic and ethanolic extracts of cinnamon were found to be equally effective against most of the experimental microorganisms, the only exception being *Pseudomonas aeruginosa*. Both extracts showed the highest inhibitory action against *Salmonella* Typhi. Vyas *et al* (2015) [12], recorded the antibacterial activity of methanolic extract of cinnamon against *S. aureus*, and *E. coli* and antifungal activity against *C. albicans* which is in harmony with the result obtained in this study. Similarly, Tomar *et al* (2015) [32] reported ethanolic extract of cinnamon to be effective against *E. coli*, *S. aureus,* and *C. albicans*, Al-Mariri *et al* (2014) [33] noted decent antibacterial property of the ethanolic extract and the essential oil of cinnamon against *Proteus* species. According to the study performed by Abdelfadel *et al* (2016) [34], water extract of cinnamon has potential antibacterial property against *Salmonella* species.

Similarly, the methanolic and ethanolic extracts of clove were found to be effective against all of the microorganisms used in the experiment. Methanolic extract of clove showed higher inhibition against *Proteus mirabilis*, whereas the ethanolic extract was found to be effective against *Salmonella* Typhi. The one-way ANOVA ($p<0.05$) of methanolic extract demonstrated it to be more efficient than Gentamicin against *Pseudomonas aeruginosa*. ANOVA ($p<0.05$) argued that there is a difference between the treatment but the post hoc test shows there is no difference between ethanolic extract and gentamicin. So, it has the same lethal effect as gentamicin. Lopez *et al* (2005) [35] reported clove oil to be efficacious against foodborne bacteria viz *S. aureus*, *E. coli*, *P. aeruginosa,* and *Salmonella* species. Also, Karuppiah *et al* (2012) [36], found that the alcoholic extract of clove has an antimicrobial effect against *P. mirabilis*. The study performed by Pinto *et al* (2009) [37],

noticed the essential oil of the clove has an antifungal effect against *C. albicans*.

Additionally, the methanolic extract of pepper was found to be inhibitory against all of the bacterial strains but not to the fungal strain (*C. albicans*), showing the highest activity against *S. aureus*. On the contrary, the ethanolic extract was found to be effective only against *E. coli* and *S. aureus* at higher concentrations. The study performed by Joshi *et al* (2009) [38] and Dahal *et al* (2017) [24] reported the antimicrobial effect of pepper methanolic extracts against *E. coli*, *S. aureus*, *S.* Typhi, *P. aeruginosa,* and *Proteus* species which coincides with the result obtained by our study.

The phytochemical analyses (**Table 4**) of the spices confirm the presence of bioactive compounds as Polyphenols, Flavonoids, and Tannins which in terms are responsible for antimicrobial, anti-inflammatory, and anti-cancer activities. The phytochemicals of the test spices correspond to that reported by Abdelfadel et al (2016) [34], Yang et al (2012) [39], Al-Numair et al (2007) [40], Gupta (2013) [41], Mishra et al (2014) [42], and Zhang et al (2014) [23]. The phytochemicals and their derivatives are responsible for the antibacterial and antifungal activities of the plant extracts. Methanol (Polarity index = 6.6) being highly polar than ethanol (Polarity index = 5.2) is responsible for a higher yield of phytochemicals from the spices [43]. Therefore, the methanolic extracts of the spices demonstrated higher antimicrobial properties with the experimental microorganisms than that of ethanolic extracts. Cinnamaldehyde, eugenol, linalool, eugenyl acetate, and cinnamyl acetate present in cinnamon is responsible for its antimicrobial property [35]. According to Gupta et al (2015) [44], a higher concentration of eugenol, β-Caryophyllene, and eugenyl acetate are responsible for antibacterial and antifungal activities of clove. Vashist et al (2016) [45] have documented, volatile components as linalool-8-mono terpentriol-3, 7-dimethyl 1-octane-3,6,7-triol, trans-cinnamic acid, etc are the major chemicals responsible for the antimicrobial effect of pepper.

Conclusion

The spices sample used for investigation constituted phytochemicals. The spices with a high amount of phytochemicals were found to be more effective against various pathogenic microorganisms whereas those spices with little phytochemicals were not as effective as those with higher phytochemicals. This result reveals that phytochemicals are the major constituents of medicinal plants that are responsible for their antimicrobial property.

Author's contribution

BA conceptualized the research proposal, performed lab works, scoring, and data analyses. PKS and RK supervised the research activities and supported data analyses and interpretations. All authors read and approved the final manuscript.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Funding

National Food Research Centre (NFRC) partially funded this research works.

Acknowledgements

The authors would like to express high lexis of thanks to **Mr. Pravin Ojha**, Scientist and **Mr. Ujjwol Subedi**, Technical Officer, and other supportive staffs of National Food Research Centre (NFRC), Nepal Agricultural Research Council (NARC) for their invaluable support and cooperation.

Ethical approval and consent

Not Applicable

References

1. Shakya AK. Medicinal plants: Future source of new drugs. Int. J. Herb. Med. 2016; **4** (4): 59-64. https://doi.org/10.13140/RG.2.1.1395.6085
2. McGaw LJ, Jäger AK, van Staden J. Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. J Ethnopharmacol. 2000;72(1-2): 247-263. doi:10.1016/s0378-8741(00)00269-5
3. Kumar Y, Agarwal S, Srivastava A, Kumar S, Agarwal G, Khan MZA. Antibacterial activity of Clove (*Syzygium aromaticum*) and Garlic (*Allium sativum*) on different pathogenic bacteria. Ind. J. Pure App. Biosci. 2014; **2**(3): 305-311.
4. Singh G, Kapoor IP, Singh P, de Heluani CS, de Lampasona MP, Catalan CA. Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of Zingiber officinale. Food Chem Toxicol. 2008;46(10): 3295-3302. doi:10.1016/j.fct.2008.07.017
5. Javid T, Adnan M, Tariq A, Akhtar B, Ullah R, AbdElsalam NM. Antimicrobial activity of three medicinal plants (*Artemisia indica*, *Medicago falcata,* and *Tecoma stans*). Afr. J. Tradit. Complement. Altern. Med. 2015; **12(3)**: 91-96. https://doi.org/10.4314/ajtcam.v12i3.11
6. Venkatachalam D, Rahman A, Sunny B, Jacob J, Kuriyan N, Raman R, Vaniapurackal R. Screening of Antimicrobial Activity of Various Extracts of the Stem *Justicia gendarussa*. Asian Journal of Research in Medical and Pharmaceutical Sciences. 2019; 6(4): 1-7.
7. Shihabudeen MSS, Priscilla H, Thirumurugan K. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. 2010; **1(10)**: 430-434.
8. Elnourani EA. Antimicrobial and Phytochemical Analyses of Selected Medicinal Plant from Kenya. Master's Degree University of Nairobi, Kenya. 2016.
9. Opinde HR. Antimicrobial Activity And Qualitative Phytochemical Composition of Crude Extracts From Medicinal Plants Against Selected Enteric Bacterial Pathogens and *Candida albicans*. [dissertation]. Kenya , Kenyatta University; 2016.
10. Nguta JM, Mbaria JM, Gakuya DW, Gathumbi PK, Kabasa JD, Kiama SG. Biological screening of Kenyan medicinal plants using

Artemia Salina L. (Artemiidae). Pharmacologyonline. 2011; **2**: 458-478. https://doi.org/10.3389/fphar.2011.00030
11. Das M, Mandal S, Mallick B and Hazra J. Ethnobotany, Phytochemical and Pharmacological Aspects of Cinnamomum zeylanicum Blume. Int Res J Pharm. 2013; 4 (4): 58-63.
12. Vyas P, Suthar A, Patel D, Dayma P, Raval J, Joshi D. Antimicrobial activity of extracts of Cinnamomum zeylanicum bark and its combination with antibiotics against various microorganisms. J Chem Pharm Res. 2015; **7** (4): 68-70.
13. Nabavi SF, Lorenzo AD, Izadi M, Sanchez ES, Daglia M, Nabavi SM. Antibacterial Effects of Cinnamon: From Farm to Food, Cosmetic and Pharmaceutical Industries. Nutrients. 2015; **7:** 7729-7748. https://doi.org/10.3390/nu7095359
14. Blaszyk and Holley (1998): Blaszyk M, Holley RA. Interaction of monolaurin, eugenol, and sodium citrate on the growth of common meat spoilage and pathogenic organisms. Int J Food Microbiol. 1998 Feb 17; 39(3):175-83. doi: 10.1016/s0168-1605(97)00134-7. PMID: 9553796.
15. Bowles BL, Sackitey SK, and Williams AC. Inhibitory effects of flavor compounds on Staphylococcus aureus, Staphylococcus aureus WRRC B124. J Food Saf. 1995; 15: 337-347.
16. Helander IM, Alakomi HL, Kala KL, Sandholm TM, Pol I, Smid EJ, Gorris LGM and Wright AV. Characterization of the Action of Selected Essential Oil Components on Gram-Negative Bacteria. J Agric Food Chem. 1998; 46: 3590-3595.
17. (Trongtokit et al 2005): Trongtokit Y, Rongsriyam Y, Komalamisra N, Apiwathnasorn C. Comparative repellency of 38 essential oils against mosquito bites. Phytother Res. 2005 Apr; 19(4): 303-9. doi: 10.1002/ptr.1637. PMID: 16041723.
18. Saeed S and Tariq P (2008). In Vitro Antibacterial Activity of Clove against Gram-Negative Bacteria. Pak J Bot. 40 (5): 2157-2160.
19. Banerjee S, Panda CK, Das S. Clove (Syzygium aromaticum L.), a potential chemopreventive agent for lung cancer. Carcinogenesis. 2006 Aug; 27(8): 1645-54. doi: 10.1093/carcin/bgi372. Epub 2006 Feb 25. PMID: 16501250.
20. Pulikottil SJ and Nath S. Potential of clove of Syzygium aromaticum in development of a therapeutic agent for periodontal disease. A review. SADJ. 2015; 70 (3): 108-115.
21. Joshi S, Gyawali A. Phytochemical and Biological Studies on Zanthoxylum Armatum of Nepal. Journal of Nepal Chemical Society. 2013; 30: 71-77. https://doi.org/10.3126/jncs.v30i0.9339
22. Vashist H, Sharma RB, Sharma D, Upmanyu N. Pharmacological activities on *Zanthoxylum aramatum*: A Review. World J Pharm Pharm Sci. 2016; **5** (12), 408-423.
23. Zhang Y, Luo Z, Wang D, He F, Li D. Phytochemical profiles and antioxidant and antimicrobial activities of the leaves of *Zanthoxylum bungeanum*. The Scientific World Journal. **2014;** 1-13. https://doi.org/10.1155/2014/181072
24. Dahal T, Kandel S, Pandeya A, Pokhrel B. Study of antibacterial and antioxidant activities of four common Nepalese kitchen spices. Afr J Pharm Pharmacol. 2017; **11** (6): 103-107. https://doi.org/10.5897/AJPP2016.4615
23. Jouda MM. The Antibacterial Effect of Some Medicinal Plant Extracts and their Synergistic Effect with Antibiotic and Non-antibiotic Drugs. Master's Islamic University-Gaza, Palestine. 2013.
24. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World Journal of Agricultural Sciences: WJAS. 2008; **4 (S)**: 839-843.
25. Ganesh P, Kumar RS, Saranraj P. Phytochemical analysis and antibacterial activity of pepper (Piper nigrum L.) against some human pathogens. Cent. Euro. J. Exp. Bio. 2014; **3 (2)**: 36-41.
26. Dimitrijević DS, Kostic DA, Stojanovic GS, Mitic SS, Mitic MN, Dordevic AS. Phenolic composition, antioxidant activity, mineral content, and antimicrobial activity of fresh fruit extracts of *Morus alba* L. J. Food and Nutr. Res. 2014; **53**: 22-30.
27. Bhattarai S, Subedi U, Bhattarai U, Karki R, Ojha P. Study on Chemical and Bioactive Components of Different Floral Sources' Honey in Nepal. Journal of Food Science and Technology Nepal. 2019; 11: 51-9. https://doi.org/10.3126/jfstn.v11i0.29690
28. Mahdavi R, Nikniaz Z, Rafraf M, Jouyban A. Determination and comparison of total polyphenol and vitamin C contents of natural fresh and commercial fruit juices. Pak J Nutr. 2010; **9** (10): 968-972. https://doi.org/10.3923/pjn.2010.968.972
29. Samatha T, Shyamsundacharya R, Srinivas P, Swamy NR. Quantification of total phenolic and total flavonoid contents in extracts of *Oroxylum indicum* L. Kurz. Asian J Pharm Clin Res. 2012; **5** (4): 177-179.
30. Faleye OS, Dada EO. Studies on the Phytochemical and Proximate Properties of the Extract of Unripe *Annona muricata* (Linn.) Fruit. Journal of Scientific Research and Reports. 2018; 20(5): 1-9. https://doi.org/10.9734/JSRR/2018/21584
31. Polshettiwar SA, Ganjiwale RO, Wadher SA, Yeole PG. Spectrophotometric estimation of total tannins in some ayurvedic eyedrops. Ind. J. Pharm. Sci. 2007; **69**(4): 574-576. https://doi.org/10.4103/0250-474X.36949
32. Tomar RS, Shrivastava V. In-vitro efficacy evaluation of ethanolic extract of *Cinnamomum zeylanicum* (Cinnamon) as potential antimicrobial agent against clinical isolates. Int J Pharm Sci Res. 2015; **6** (5): 2201-2204.
33. Al-Mariri A, Safi M. In-vitro antibacterial activity of several plant extracts and oils against some gram-negative bacteria. Iran J Med Sci. 2014; 39 (1): 36-43.
34. Abdelfadel, MM; Khalaf, HH; Sharoba, AM; Assous, MTM. Effect of extraction methods on antioxidant and antimicrobial activities of some spices and herbs extracts. Journal of Food Technology and Nutritional Sciences. 2016; 1: 1-14
35. Lopez P, Sanchez C, Batlle R, Nerin C. Solid- and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. J Agric Food Chem. 2005; **53** (17): 6939-6946. https://doi.org/10.1021/jf050709v
36. Karuppiah P, Rajaram SK. Antibacterial effect of *Allium sativum,* cloves, and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens. Asian Pac J Trop Biomed. 2012; **2** (8): 597-601. https://doi.org/10.1016/S2221-1691(12)60104-X
37. Pinto E, Silva LV, Cavaleiro C, Salgueiro L. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on Candida, Aspergillus, and dermatophyte species. J Med Microbiol. 2009; **58**: 1454-1462. https://doi.org/10.1099/jmm.0.010538-0
38. Joshi B, Lekhak S, Sharma A. Antibacterial property of different medicinal plants: *Ocimum sanctum*, *Cinnamomum zeylanicum*, *Xanthoxylum armatum,* and *Origanum ajorana*. J Sci Eng Technol. 2009; **5** (1): 143-150. https://doi.org/10.3126/kuset.v5i1.2854
39. Yang CH; Li RX; Chuang LY. Antioxidant activity of various parts of *Cinnamomum cassi*. Molecules. 2012; **17**: 7294-7304. https://doi.org/10.3390/molecules17067294
40. Al-Numair KS., Ahmad D, Ahmad SB, Abdullah, Al-Assaf H. Nutritive value, levels of polyphenols and anti-nutritional factors in Sri Lankan cinnamon (*Cinnamomum zeyalnicum*) and Chinese cinnamon (*Cinnamomum cassia*). Res. Bult., 2007; 154: 5-21.
41. Gupta D. Comparative analysis of spices for their phenolic content, flavonoid content, and antioxidant capacity. American International Journal of Research in Formal, Applied & Natural Sciences. 2013; 4: 38-42.
42. Mishra RP, Sharma K. Antimicrobial activities of *Syzigium aromaticum* L. (Clove). Int Res JBiological Sci. 2014; **3**(8), 22-25.
43. Vargas RA, Malacara CFP, Petricevich VL. Characterization of chemical compounds with antioxidant and cytotoxic activities in *Bougainvillea x buttiana* Holttum and Standl, (var. Rose) Extracts. Antioxidants. 2016; **5** (45), 1-11. https://doi.org/10.3390/antiox5040045
44. Gupta D, Girija. Evaluation of in vitro antioxidant and antimicrobial activities of various spices of Indian origin. International Journal of Pharmacy and Pharmaceutical Sciences. 2015; **7**(8); 137-141.
45. Vashist H, Sharma RB, Sharma D, Upmanyu N. Pharmacological activities on *Zanthoxylum aramatum*: A Review. World J Pharm Pharm Sci. 2016; **5** (12), 408-423.