Original Article

Preliminary Studies on some Nepalese Kitchen Spices for their Antibacterial Activities

Abstract

The main objective of the present study was to evaluate the antibacterial activity of nine Nepalese kitchen spices viz Clove (Eng.), Lwang (Nep)- Syzygium aromaticum, Myrtaceae. Cinnamon (Eng), Dalchini (Nep)Cinnamomum zeylanicum, Lauraceae, Black piper (Eng), Marich (Nep), Piper nigrum, Piperaceae, Long piper (Eng), Pipla (Nep) Piper longum, Piperaceae, Trigonella (Eng), Methi (Nep)Trigonella foenum graecum, Fabaceae and four others spices Trachyspermum (Eng), Jwano (Nep) Trachyspermum ammi, Coriandrum (Eng), Dhaniya (Nep) Coriandrum sativum, ,Cumin (Eng), Jira (Nep) Cuminum cyminum, and Fennel (Eng), Souf (Nep)Foeniculum vulgare, from Umbelliferae.

The value of foreign organic matter was evaluated and found to be highest in Coriandrum sativum (6.2%) whereas lowest in Piper longum (0.8%). Ethanolic. extract of Fennel at 25 mg/ml concentration shows the strongest sensitivity for five bacteria (E. coli- 19.21 mm, S. typhi- 12.61 mm, S. paratyphi- 12.86 mm, Pseudomonas aeruginosa- 17.03 mm and Staphylococcus aureus- 18.12mm). Similarly, Trachyspermum ammi 50 mg/ml concentration showed zone of inhibition (13.81 mm) against S. paratyphi.

Key words: Kitchen Spices, Extract, Zone of Inhibition, Antimicrobial activity,

Dharma Prasad Khanal, Bechan Raut*, Kiran Sundar Bajracharya* and Raj Kumar Karki***

**Manmohan Memorial Institute of Health Sciences, Nakhhu, Lalitpur, Nepal*

***Lomus Pharmaceutical Private Limited, Kathmandu, Nepal*

Corresponding Author: Dharma Prasad Khanal,Manmohan Memorial Institute of Health Sciences, Nakhhu, Lalitpur, Nepal

email: drdpk@yahoo.com

Introduction

Spices and condiments are defined as "Vegetable products or mixtures, free from extraneous matter, used for flavoring, seasoning or imparting aroma in foods." Spices are used for flavor, color, aroma and preservation of food or beverages. Spices may be derived from many parts of the plant: Bark, roots, rhizomes, leaves, fruits, seeds, flowering buds, aerial parts and resin. Spices come from the bark (cinnamon), root (ginger, onion, and garlic), buds (cloves, saffron), seeds (yellow mustard, poppy, and sesame), berry (black pepper), or the fruit (allspice, paprika) of tropical plants and trees [1]. The Values of spices are food additive for the purpose of flavor, color or taste to the food. As a preservative that kill harmful bacteria or prevents their growth and prolong the storage life of foods by preventing rancidity through their antioxidant activity.

It is used as a stimulant of the appetite and increase the secretion and flow of gastric juices and a household medicine - for the alleviation of coughs and pharynx complaints, gastritis and dyspepsia, flatulence, rheumatism, bronchitis etc. The medicinal value of many spices used in Nepalese kitchen has been known for centuries. There are around 40 to 50 spices of global economic and culinary importance. There are also many other species that are used in traditional cooking in the region of their natural occurrence but have yet to reach any significant trade. [1].

Spice and herb plants do not require large land areas for profitable cultivation and can also be gathered as many spice and herb plants can be produced with minimal inputs of cash, labor and land. From the wild. In the case of cultivation, growing can be achieved without excessive investments.

The genus Cinnamomum comprises several hundred species, which occur in Asia and Australia. These are evergreen trees and shrubs and most of the species are aromatic. C. zeylanicum, the source of cinnamon bark and leaf oils, is a tree indigenous to Sri Lanka. Manyspecies of cinnamon yield a volatile oil on distillation [2-4].

ISSN: 2091-1041 | VOLUME 1 | ISSUE 2 |2013

The volatile oil of buds of C. zeylanicum contains α-bergamotene (27.4%) and α-copaene (23.1%) as the major compounds [4], Cinnamon species very successfully be used against the food spoiling bacteria E. coli. Coliform bacteria are easily encountered in water. Cinnamomum zeylanicum essential oil showed stimulatory effects on macrophages. Phagocytosis and killing of invading microorganisms by macrophages constitute the body's primary line of defence against infections [5].

Trigonella foenum-graecum (Family Fabaeace) is called methika in Ayurveda and used as medicine for the treatment of wounds, abscesses, arthritis, bronchitis and digestive disorders etc since oldest time. It is also eaten in winters as to improve immunity and protects heart, brain and other vital organs of body through its medicinal properties. Fenugreek (Trigonella foenum-graecum L.) is an annual legume seed having 3-5 mm X 2-3 mm size rectangular to oval in shape with deep grooves between the radical and cotyledon,Varies in color form pale brown to golden yellow mainly used as a spice crop in many parts of the world. 3-Hydroxy-4,5-dimethyl-2(5H)-fbranone (sotolone) was established as the character impact flavor compound of fenugreek on the basis of gas chromatography-olfactometry [5].

Long pepper (Piper longum),(Pippali), is a flowering vine , cultivated for its fruit, which is usually dried and used as a spice and seasoning. Long pepper has a similar, but hotter, taste usually dried and used as a spice and seasoning [6]. Coriander (Coriandrum sativum), is an annual herb in the family Apiaceae. Coriander is native to regions spanning from Southern Europe and North Africa to South Western Asia. Coriander consists of dried ripe fruits of Coriandrum sativum Linn. (Fam. Umbelliferace) ; a slender, soft, hairless, glabrous, branched, annual and a perennial herb growing to 50 centimeters (20 in) tall cultivated all over India, giving characteristic aroma when rubbed; crop matures in 2-3 months after sowing; herb is pulled out with roots, after drying, fruits threshed out and dried in sun .The fruit is a globular, dry schizocarp 3-5 mm (0.12-0.20 in) in diameter. The small-fruited types contain higher oil (0.5-2%), which is extracted for its essential oil while the larger fruit with a lower oil (<1%), is used for grinding and blending. The fresh leaf (cilantro) is used in Asian cuisine. [7].

The fruit of Trachyspermum ammi consists of two mericarps, grayish brown, ovoid, compressed, about 2 mmlong and 1.7 mm wide, 5 ridges and 6 vittae in each mericarp, usually separate, 5 primary ridges. seed' (i.e., the fruit pod, 2 mm long and 1.7 mm wide, 5 ridges) is often confused with lovage seed; even some dictionaries mistakenly state that comes from the lovage plant. An online search for lovage seeds finds many stores calling their ajwain seeds lovage [8] Pepper (Piper nigrum) is a perennial vine, which produces a small berry fruit, which is dried to become pepper. Pepper is a plant of the humid tropics requiring adequate rainfall and warmth for its growth. It is grown successfully between latitudes of 20° North and 20° South and from sea level up to an altitude of 2400 m. The crop can tolerate a temperature range between 10° and 40°C but the optimum is between 25°C-40°C. A well-distributed rainfall in the range of 1250 mm -2000 mm is considered necessary for pepper production [9] The clove tree (Syzygium aromaticum) is an evergreen which grows up to 15m in height. The clove tree produces buds which are used whole or ground as a spice. Bud and stem oils and oleoresins, and leaf oil, are used principally as a source of eugenol. [9]

Cumin (Cuminum cyminum), a small annual herb native to the Mediterranean region, which is grown for its aromatic dried fruit that is widely used in cooking. Cumin prefers areas with low atmospheric humidity during the period of flowering, seed formation and ripening, Fruit is of light brown color with inch ¼ in length. The apex and base are thin and bears strip all over it. [9].

Considering the large number of different groups of chemical compounds present in essential oils, it is most likely that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell. Antimicrobial sensitivity test of Nepalese medicinal plants has been studied but focused on kitchen spices are not done so far as our literature survey could ascertain. [11-14]

Mode of antibacterial action of essential oils is widely discussed in the research literature. Not all of these mechanisms are separate targets; some are affected as a consequence of another mechanism being targeted. An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable. Leakage of ions and other cell contents can then occur. Although a certain amount of leakage from bacterial cells may be tolerated without loss of viability, extensive loss of cell contents or the exit of critical molecules and ions will lead to death [15]

Material and Method

Collection and identification of samples All the samples were collected from street shop located near by the Lagankhel bus park, Lalitpur, Nepal. The specimens were identified on the basis of the apparent morphological characters of the spices [16].

Processing of spices' samples

First of all the plant materials were cleaned and separated the foreign organic matter (FOM). They were air dried and grinded so as to obtain the powder with the help of grinder.

Preparation of extract

20 gm sample of each ground sample were soaked in 100 ml of absolute ethanol for 24 hours and mixed in a magnetic stirred for 30 min. The solution was filtered to get extract through the whatman numberone filter paper. The filtrate was concentrated in rotatory evaporator at 70^{o}C. The extract was measured to determine their extractive value.

Collection and maintenance of culture

All the test organisms were collected from Patan Academy of Health Sciences, Patan, Lalitpur. These organisms were first inoculated into sterile nutrient broth to revive, then were subculture and were tested for their purity by examining their morphological characteristics (by gram staining tests) and biochemical tests. Purely isolated colony of each organism was incubated at 37^{0}C for 4 hours to bring them into log phase.

Preparation of working solution of crude extracts

Working solution was prepared by dissolving the crude extract of each spice in ethanol to prepare the concentration of 100mg/ml, 50 mg/ml and 25 mg/ml.

The in-vitro antimicrobial activity of spices' extracts in 25 mg/ml, 50 mg/ml and 100 mg/ml concentrations of each spices' extract were subjected for in vitro antimicrobial tests by agar well diffusion method to screen and evaluate them with the determination of zone of inhibition (ZOI).

Determination of zone of inhibition

Muller Hilton agar was prepared in 90 mm Petridis with thickness of about 4 mm. Sterile cotton swab was used to inoculate the entire surface of agar plate and the inoculums was allowed to dry for 5-15 min in the laminar flow. With the help of sterile borer (diameter, d=6mm), 4 wells were made in plates and labeled. To each of the labeled well, the different concentration (25 mg/ml, 50 mg/ml and 100 mg/ml) of extract suspension of respective spices was dispensed in the amount of 20 micro liters with the help of micropipette. In the fourth well the ethanol was placed to control the sensitivity of the microorganism. For each organism, antibiotic disc was placed in the same condition for control. The test was performed in duplicates for the confirmation [17]

A variety of laboratory methods can be used to measure the in vitro susceptibility of bacteria to antimicrobial agents. The most widely used test methods to measure the antibacterial activity of essential oils and their constituents are Disk diffusion, Agar wells, Agar dilution method and Broth dilution method. The pharmacological effects of these spices have been investigated. P.longum when orally administered to 20 children significantly decreased the frequency and severity of attacks of bronchial asthma [18]. Most of the antimicrobial activity in spices is due to essential oils that appear to derive from oxygenated terpenoids, particularly phenolic terpenes, phenylpropanoids and alcohols. Other constituents (e.g., hydrocarbons) that typically showed low activities can be used in combinations to increase their bioactivities [19].

ISSN: 2091-1041 | VOLUME 1 | ISSUE 2 |2013

Results and discussion

S.N.	Sources	Parts used	FOM value (%)	FOM
1	Cinnamomum zeylanicum	Bark	2.5	Moss, other barks, straw
2	Syzygium aromaticum	Buds	1.6	Stalk of clove, dust
3	Coriandrum sativum	Fruits	6.2	Insect attack, dust, straw
4	Foeniculum vulgare	Fruits	4.1	Insect attack, dust, straw
5	Trigonella foenum-graecum	Seeds	1	Dust
6	Cuminum cyminum	Fruits	1.6	Straw, dust
7	Piper nigrum	Fruits	2.3	Dust, other fruits
8	Piper longum	Fruits	0.6	Dust, other fruits
9	Trachyspermum ammi	Fruits	1.3	Dust, other fruits, straw

Table -2: Antimicrobial Test

S.N.	Spices	S aureus	E Coli	S typhi	S paratyphi	P aeruginosa	S pyogens
1	Cinnamomum zeylanicum	+	+	+	+	+	+
2	Syzygium aromaticum	+	+	+	+	+	+
3	Coriandrum sativum	-	-	-	-	-	-
4	Foeniculum vulgare	+	++	+ +	+	+	-
5	Trigonella foenum-graecum	-	-	-	-	-	-
6	Cuminum cyminum	+	+	+	+	+	+
7	Piper nigrum	+	+	+	+	+	+
8	Piper longum	+	+	-	-	-	-
9	Trachyspermum ammi	-	-	-	-	-	-

+= <14.90 mm and ** = for >15 mm

Foeniculum vulgare (Apiaceae) exhibit antifungal, antibacterial, antioxidant, antithrombotic and hepatoprotective activities, lending support to the rationale behind several of its therapeutic uses. The essential oil extracted from the fruits of F. vulgare exhibited antibacterial effect against food borne pathogens such as Escherichia coli, Bacillus megaterium and Staphylococcus aureus, E. coli, Listeria monocytogenes and S. aureus [19-20]. These findings also supported by our present study in relation to S aureus and E Coli.

Conclusion

The value of foreign organic matter was found to be highest in Coriandrum sativum (6.2%) whereas lowest in Piper longum (0.8%). .Fennel extract at 25 mg/ml concentration shows the higest sensitivity for five bacteria (E. coli- 19.21mm, S. typhi- 12.61 mm, S. paratyphi- 12.86 mm, Pseudomonas aeruginosa- 17.03 mm and Staphylococcus aureus- 18.12mm). Similarly, Trachyspermum ammi 50 mg/ml concentration showed zone of inhibition (13.81 mm) against S. paratyphi. The ZOI of antibiotic disc (30 mcg ciprofloxacin and chloramphenicol) for the tested bacteriais greater than the sensitivity of respective spices. According to our study fennel as a kitchen spice may be the candidate for further investigation.

Acknowledgement

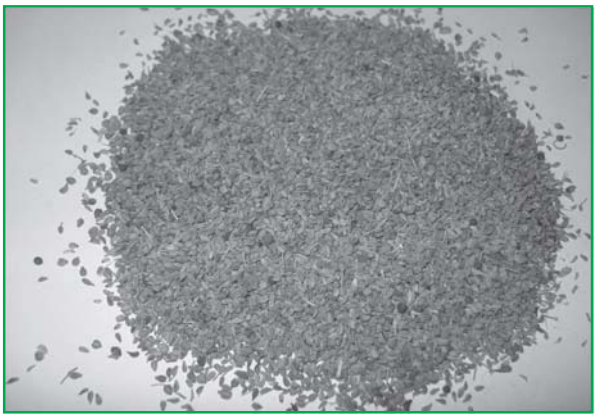
We would like to express our appreciation and thanks to University Grant Commission Nepal for providing the research grant, Lomus Pharmaceutical Pvt. Ltd. Gothatar, Kathmandu for the required instruments and Patan Academy of Health Sciences, Lalitpur, for the required micro-organisms.

References

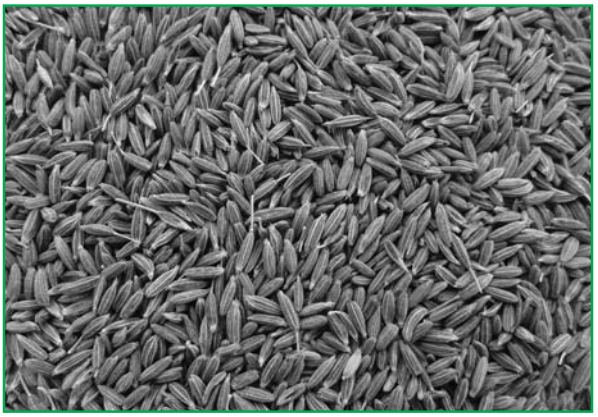
1. Green, C., J. Steele, and J. Willemse- The development and prospects for minor industrial crops in selected Southern African countries. World Bank Technical Paper, 1999(No. 424): p. 139-166.
2. CBI Market Information Database o URL: www.cbi.eu
3. Guddadarangavvanahally K. Jayaprakasha, Lingamallu Jaganmohan Rao and Kunnumpurath K. Sakariah- Chemical Composition of Volatile Oil from Cinnamomum zeylanicum Buds. Z. *Naturforsch*. 2002;57: 990-993.
4. Pritam D. Nimje, Hemant Garg, Anu Gupta, Niharika Srivastava, Monica Katiyar and C. Ramalingam - Comparison of antimicrobial activity of Cinnamomum zeylanicum and Cinnamomum cassia on food spoilage bacteria and water borne bacteria. *Der Pharmacia Lettre,* 2013; 5 (1):53-59.
5. Rashmi Yadavi, Richa Tiwari, Partha Chowdary, Chandan Kumari Pradhan-A Pharmacognostical Monographa of Trigonellafoeum-graecum Seeds: *International Journal of Pharmacy and Pharmaceutical Sciences,* 2001,Vol 3, Suppl 5,.
6. Lakshmi Arambewela & Ruvina Silva - Srilankan Medicinal Plant Monographs and Analysis, 1999 Volume 3, Piper Longum, (Cylon Institute of Scientific and Industrial Research) 363, Buddhaloka Mawatha, Colombo 7.
7. Rashmi Yadavi, Richa Tiwari, Partha Chowdary, Chandan Kumari Pradhan-A Pharmacognostical Monographa of Trigonellafoeum-graecum Seeds: *International Journal of Pharmacy and Pharmaceutical Sciences,* 2001,Vol 3, Suppl 5.
8. Verma Anju, Dr.S.N.Pandeya, , Sanjay Kumar Yadav,Dr. Styawan Singh, Pankaj Soni - A Review on Coriandrum sativum (Linn.): An Ayurvedic Medicinal Herb of Happiness. *Journal of Advances in Pharmacy and Healthcare Research*, 2011;1(3)
9. Dhirendra Singh, S P Choudhary - Evaluation of ajowain (Trachyspermum ammi L.) genotypes suitable for semi arid regions. *J of Spices and Aroma,* 2008, Vol 17, No 2.
10. FAO and UNIDPO- Herbs, spices and essential oils Post-harvest operations in developing countries (2005).
11. Imre Blank, Jianming Lin, StCphanie Devaud, Rend Fumeaux, and Laurent B. Fay - Principal navor Components of Fenugreek, Chapter -3, Nestec Limited, Nest16 Research Center, Vers-chez-les-Blanc, P.O. Box 44, CH-1000 Lausanne 26, Switzerland.
12. Beuchat LR and Golden DA- Antimicrobials occurring naturally in foods. Food Technologies, 1989, 43: 134-142.
13. Devkota, K.P., Acharya, R., Baral, M.P. and Pokharel, R.P - Antimicrobial Activity of Some Herbal Plants Used in Traditional Medicine in Nepal. Proceedings, Third National Conference on Science & Technology, RONAST, II : 2000 . 1311-1317.
14. Devkota, K.P. and Dutta, I.C. 2001 - Antibacterial activities of commercially traded herbs used in traditional medicines in communities of Doti district, Nepal, Report submitted to IUCN, Kathmandu, Nepal.
15. Dutta, I.C. & Karn, A.K. - Antibacterial Activities of Some Traditionally Used Medicinal Plants of Daman, Nepal. 2007. TU, IOF & ComForm, Pokhara, Nepal.
16. S N Pandey & A Chandha.- A Text Book of Botany, 1994, Vol-II, Vikash Publishing House Ltd. New Delhi India
17. Roopashree TS, Raman Dang , Shobha Rani RH Narendra C- Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis. *International Journal of Applied Research in Natural Products* 2008;1(3):20-28.
18. Sara Burt - Essential oils: their antibacterial properties and potential applications in foods-a review- *International Journal of Food Microbiology* 2004;94:223-253.
19. Dananukar, S. A., Karandikar, S. M., Desai, M - Efficacy of Piper Longum in Childhood Asthma, *Indian Drugs,* 1984; 21 (19): 384-388.
20. Manzoor A. Rather , Bilal A. Dar a, Shahnawaz N. Sofi Bilal A. Bhat Mushtaq A - Qurishi- Foeniculum vulgare: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety: *Arabian Journal of Chemistry* (2012)

ISSN: 2091-1041 | VOLUME 1 | ISSUE 2 |2013

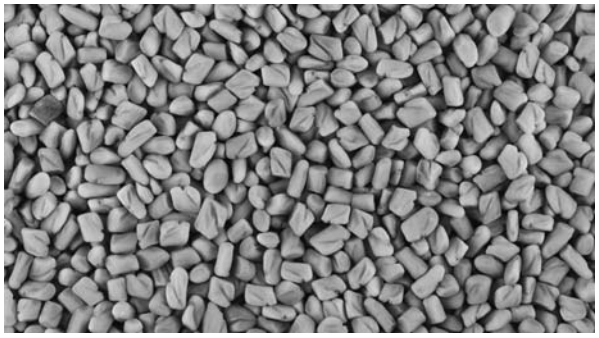
Annex-I: Spices



Trachyspermum (Eng)
Jwano (Nep)



Cumin (Eng)
Jira (Nep)



Trigonella (Eng), Methi (Nep)

Coriander (Eng), Dhaniya (Nep)

Cinnamon (Eng), Dalchimn (Nep)

Clove (Eng), Lwang (Nep)

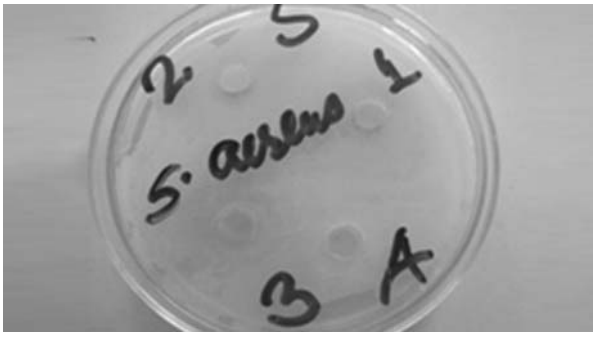
Long Piper (Eng)
Pipla (Nep)

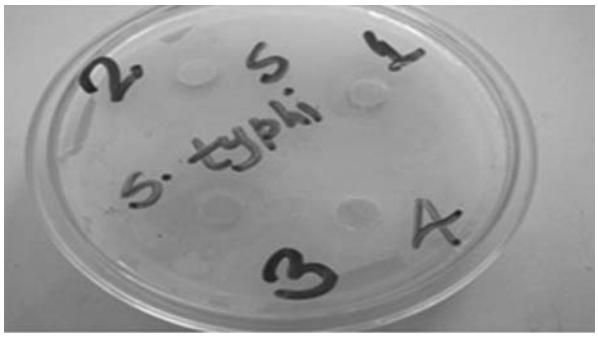
Black Piper (Eng)
Marich (Nep)

Fennel (Eng)
Souf (Nep)

Annex-II: Experimental Petri plates

S-sauf (Foeniclum Vulgare), 1-25 mg/ml, 2-50 mg/ml, 3-100 mg/ml, 4 absolute Ethanol





Annex-III: Zone Reader (Lomus Pharmaceuticals Pvt Ltd, Gothatar, Kathmandu, Nepal)

ISSN: 2091-1041 | VOLUME 1 | ISSUE 2 |2013