Research Article

Determination of Antimicrobial Properties of VIT ALL MART Spices for Pharmacological Uses

Bhumika Patel*, Sucheta Patnaik, Isha Biswas, Aishwarya Naik, Nikita Khandelwal, Suneetha V
School of Biosciences and Technology, VIT University, Vellore 632014, Tamil Nadu India, India.
***Corresponding author's E-mail:** bhumika.patel2015@vit.ac.in, vsuneetha@vit.ac.in

Received: 25-10-2016; **Revised:** 05-01-2017; **Accepted:** 18-01-2017.

ABSTRACT

Spices are known not only for their exquisite aroma and flavor but they are also rich in antioxidants and antimicrobial properties and hence are highly beneficial to human health. The project aims at focusing the use of spices as antibacterial agents. Secondary metabolites of spices like volatile oil of black pepper (*Piper nigrum*), extracted oil of cloves (*Syzigium aromaticum*), curcumin of turmeric (*Curcuma longa*), and extracts of cinnamon (*Cinnamomum zeylanicum*) possess antibacterial properties in varying concentrations. The project undertaken, describes the methodologies we have performed to assay the antimicrobial (antibacterial) properties of spices namely, cloves (*Syzigium aromaticum*), black pepper (*Piper nigrum), cinnamon (Cinnamomum zeylanicum*) and turmeric *(Curcuma longa*). Antimicrobial properties of spices at different concentration are performed to get maximum efficiency.

Keywords: Antimicrobial, Spices, *Piper nigrum*, *Syzigium aromaticum*, *Curcuma longa*, *Cinnamomum zeylanicum.*

INTRODUCTION

Anti microbial refers to a substance which works against the ill-effects of a microbe (mostly includes bacteria and fungi). Antimicrobials are popular example of antibiotics which are incessantly used these days by people, even for common cold. This excess use of antibiotics every now and then has led to the microbes developing resistance towards these antibiotics. Its action on the microbe hence ceases to work. The ultimatum lies in nature being the most powerful healer and hence man resorts to natural sources of antimicrobial agents. Spices are rich sources of antimicrobial, to be more precise, antibacterial agents. Also, Indian cuisines encompass frequent use of spices since ancient age.[1]Thus along with acting as flavoring and coloring agent for food; Spices play an important role as antibacterial agents. Humans also suffer from numerous fungal diseases which can be cured by antifungal agents. Antiseptics used for applying on wounds are also under the category of antimicrobial agents. Spices are of keen interest to researchers as it is a natural source for human wellness. Antibiotics and other antimicrobial agents have stopped working as the microbes have become resistant to these agents. Spices are used popularly for antimicrobial activity as antibiotics are failing to show their effect. The present paper aims at focusing on use of spices as antibacterial agents rather than antibiotics. Spices have humongous amounts of secondary metabolites by virtue of which they are found to exhibit antibacterial activity. This elucidates why spices can be used as a good preservatory agent and for medicinal purposes.

This paper describes the methodologies undertaken to assay the antimicrobial (antibacterial) properties of spices namely, cloves (*Syzigium aromaticum*), black pepper (*Piper nigrum), cinnamon (Cinnamomum zeylanicum*) and turmeric *(Curcuma longa*). Spice extracts of each spice were prepared by mixing equal proportions of spice and ethanol. Antibacterial effect of each spice was observed on a bacterial strain *E. coli*- DHY. Antibacterial assay of spices was performed by Agar Well Diffusion method, in which agar plate was swabbed using pre-sterilised disposable swabs with pure bacterial culture and then wells were prepared using syringe system.[12] Then, various concentrations of each spice extract was loaded into agar wells and incubated for 24-36 hours. Inhibition zones around each well were obtained. The diameters of the wells were noted down and area was calculated. The more inhibition zone area indicated higher antibacterial activity.

Results revealed that at 100% concentration of the spice cloves had the maximum area of inhibition zone followed by cinnamon, black pepper and turmeric in order. The results obtained pave way for novel medication strategies to be developed using these naturally occurring spices for the long life of humans.

MATERIALS AND METHODS

The spices namely cloves (*Syzigium aromaticum*), black pepper (*Piper nigrum), cinnamon (Cinnamomum zeylanicum*) and turmeric *(Curcuma longa)* were bought from all mart, outside gate no.2, Vellore institute of Technology, Vellore for the present study.

Preparation of spice extracts

1 gram of each spice was weighed using digital balance in lab no.216 (cell and molecular biology lab, SMV building). Each one of them were crushed and grinded using mortar and pestle. These spice powders were then mixed with 5 mL of distilled water and ethanol mixture of ratio 1:1. This extract was centrifuged at 5000 rpm for 10 minutes and

the supernatant was used as spice extract for assay of antimicrobial activity of spices[3].

Figure 1: Powdered Spice in Ethanol and water (1:1 ratio).

The microorganism

Strain of *E. coli* (*Escherichia coli)* namely *E. coli*-DHY (pure culture) obtained from Instrumental and food analysis lab of School of Bio Sciences and Technology, VIT University, Vellore.

Procedure followed

We took 4 Petri plates (borosil) and two 250ml conical flasks. Then 1.3 grams of Nutrient broth (containing Peptone, Beef extract, Sodium chloride and adjusted pH) was added in 100ml distilled water which was then made in to a solution.[10] After sterilizing the nutrient broth for 45 minutes we carefully placed the conical flask containing nutrient broth in a laminar airflow (sterilized environment) which was cleaned and wiped with ethanol, then we took an inoculum loop full of *E. coli* DHY culture and dip it in the nutrient broth kept near the burning flame, finally after sterilizing the edges of conical flask near the flame and tightly screwing the cap, we put it on a shaker incubator at 100rpm, for 24 hours.[2]

Figure 2: Incubating bacterial culture on shaker (100 rpm)

This will help the bacteria to grow properly, then we took 4 Petri plates of same uniform sizes and 1 conical flask (250ml), we took 3 grams of Mueller-Hinton agar (veg agar) and 2 grams of agar- agar (for thickening) and make a 100ml solution with water as the solvent, then we tightly closed the cap and along with Petri-plates wrapped in newspaper covers.[5] We put all the materials (including the syringe setup for making wells in the plates) in a sterilizing autoclave bag and load it for sterilization for 45 minutes.[9] After, releasing the pressure, we taken out the sterilization bag and kept it in laminar airflow(wiped clean with ethanol), then we carefully took the Petri plates, and kept them open near the flame after which we carefully add the agar in them, after adding agar, we took sterile cotton swabs, which are dipped in the nutrient broth culture and then swabbed on the surface of agar.[6]

Figure 3: Swabbing the culture on nutrient Agar Surface

Then we took the sterilized syringe connected to a pipe, and made 5 wells in all the 4 agar plates for different concentration, covered them and kept them in the laminar airflow for some time.

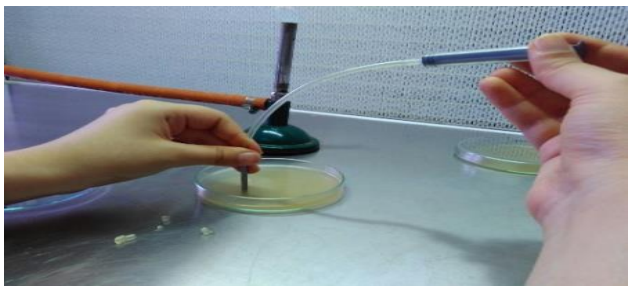

Figure 4: Making Wells in the agar gel in agar plate

Then, different concentrations of spice extracts (10%,25%,50%,75%,100%) were added in the wells (Each Petri plate had one spice extract in different concentrations).[7] Then, after adding the extracts, we labeled the petri plates and kept it in the incubator for 24 hours.[11] After, 24 hours we measured the zone of inhibition of the spice extract which appeared as clear circles and based upon the area calculated we inferred the efficiency of the spices.[8]

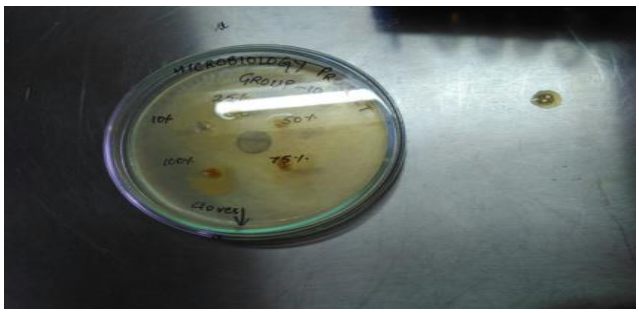

Figure 5: Wells containing spice extract of different concentrations.

OBSERVATION

Diameter of wells-0.2cm

S. no.	Spice	Radius of effect for 10% concentration	Radius of effect for 25% concentration	Radius of effect for 50% concentration	Radius of effect for 75% concentration	Radius of effect for 100% concentration
1.	Black Pepper	0.2cm	0.05cm	0.05cm	0.15cm	0.3cm
2.	Cloves	0.4cm	0.2cm	0.2cm	0.8cm	1.3cm
3.	Turmeric	0.1cm	0.2cm	0.4cm	0.1cm	0.2cm
4.	Cinnamon	0cm	0.5cm	0.5cm	0.3cm	0.65cm

RESULTS

Area of well-0.12566 cm^2

S. no.	Spice	Area of effect for 10% concentration (cm^2)	Area of effect for 25% concentration (cm^2)	Area of effect for 50% concentration (cm^2)	Area of effect for 75% concentration (cm^2)	Area of effect for 100% concentration (cm^2)
1.	Black Pepper	0.1256	0.0078539	0. 0078539	0.07068	0.28274
2.	Cloves	0.5026	0.1256	0.1256	2.0106	5.3093
3.	Turmeric	0.0314	0.1256	0.5026	0.0314	0.1256
4.	Cinnamon	0	0.7854	0.7854	0.2827	1.3273

Figure 6: Culture plates after 24 hours.

DISCUSSION

The effect of anti-microbial quantity for Black pepper is high at 10% and then analyzed at 25%, 50% and then again increases at 75% and 100%. However the overall anti-microbial effect of Black pepper is very less as compared to other spices.

The effect of anti-microbial action for Cloves is high at 10% and decreases at 25%, 50% and then again increases at 75% and 100%. The overall anti-microbial effect of Cloves is highest as compared to other spices.

The effect of anti-microbial action for Turmeric increases from at 10% to 25%, is highest at 50% and then again increases from 75% to 100%.

The effect of anti-microbial action for Cinnamon is zero at 10%, increases and remains constant at 25% and 50%, then decreases at 75% and then increases and reaches maximum at 100%.

CONCLUSION

From the experiment performed above we conclude that, Spices are quite efficient in their antimicrobial action, and as we increase the concentration, we observe that the antimicrobial efficiency also increases (100% is very efficient). Also, among the spices used, cloves have the highest anti-microbial activity and turmeric has the least antimicrobial activity.

Acknowledgement: We want to express our special thanks to Our Honorable chancellor Dr. G.Viswanathan, Vice presidents Dr. Sekar Viswanathan, Sri. Sankar Viswanathan and Sri. G.V.Selvam for constant support and encouragement.

REFERENCES

1. Pandey Bhawana, Khan Shabina and Singh Sheetal - *A Study of antimicrobial activity of some spices* International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 3, Number 3(2014), pp.643-650.
2. M.N Indu; A.A.M Hatha; C. Abirosh; U. Harsha; G. Vivekanandan - *Antimicrobial activity of some of the South-Indian spices against serotypes of Escherichia Coli, Salmonella, Listeria Monocytogenes and Aeromonas Hydrophila* Brazilian Journal of Microbiology (2006) 37, 153-158 ISSN 1517-838.
3. Oyas Ahmed Asimi, Sahu N.P, Pal A.K- *Antioxidant activity and antimicrobial property of some Indian spices* International Journal of Scientific and Research Publications, Volume 3, Issue 3, March 2013 1 ISSN 2250-3153.
4. Sundaresan Athul, Selvi Arul and Manonmani H.K- *The Anti-Microbial Properties of Triticum aestivum (Wheat Grass) Extract* International Journal of Biotechnology for Wellness Industries, 2015, vol 4, pp 84-91.
5. Thongson, C.; Davidson P.M.; Mahakarnchanakul, W.; Weiss, J. - *Antimicrobial activity of ultrasound-assisted solvent-extracted spices*. Lett. Appl. Microbiol., 39(5), pp 401-406, 2004.
6. Shelf L.A -*Antimicrobial effect of spices* Journal of Food Safety 10.1111/j.1745-4565.1984.tb00477.x,Volume 6, Issue 1, March 1984, pp 29-44.
7. Evandro Leite de Souza[I]; Tânia Lúcia Montenegro Stamford; Edeltrudes de Oliveira Lima[I]; Vinícius Nogueira Trajano; José Maria Barbosa Filho-*Antimicrobial effectiveness of spices: An approach for use in food conservation systems.* Brazilian archives of Biology and Biotechnology ISSN 1678-4324, Volume 48, no.-4, July 2005.
8. M.Melvin Joe; J.Jayachitra and M.Vijayapriya-*Antimicrobial activity of some common spices against certain human pathogens* Journal of Medicinal Plants, ISSN1996-0875© 2009 Academic Journals Volume 3, No.-11, December 2009, pp 1134-1136.
9. Arora D.S;Kaur J-*Antimicrobial activity of spices* International Journal of Microbial Agents, PMID 10461845,Volume 12, no.3, August 1999.
10. Burt Sara-*Essential oil: their antibacterial properties and potential applications in foods* International Journal of Food Microbiology Volume 4, Issue 3, August 2004, 223-253.
11. Laura l.Zaika-*Spices and herbs: their antimicrobial activity and its determination,* Journal of Food Safety, 10.1111/j.1745-4565.1988.tb00511.x,Volume 9, Issue 2, July 1988, Pp 97-118.
12. Bin Shan; Yi-Zhong Cai; John D. Brooks; Harold Corke *The In-vitro antibacterial activity of dietary spice and medicinal herb extracts* International Journal Of Food Microbiology, Volume 117, Issue 1,June 2007,pp 112-119.
13. Palmer Smith; Stewart and Fyfe *Antimicrobial properties of Plant essential oils and essences against five important food borne pathogens* 10.1046/j.1472-765X.1998.00303.x, volume 26, Issue 2, February 1998, pp 118-122.

Source of Support: Nil, **Conflict of Interest:** None.