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Phytochemical analysis of Cinnamomum zeylanicum for antibacterial activity against B.subtilis

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Abstract: Based on Ethno botanical knowledge, an attempt has been made to evaluate the antibacterial properties of Cinnamomum zeylanicum (Dalchini), against medically important bacterial strain, namely Bacillus subtilis. Disc diffusion method has been used to evaluate antibacterial activity of methanol extracts. The plant extract was active against bacteria B. subtilis. Methanol extract of Cinnamomum zeylanicum gave its maximum size of zone of 25mm in case of Bacillus subtilis (0.5gm/ml). Therefore, Cinnamomum zeylanicum can be selected for further investigation to determine their therapeutic potential. For development of new pharmaceuticals this plant can be used to discover bioactive natural products.
Keywords: Phytochemical analysis, antibiogram and plant extract.

I. INTRODUCTION

Excessive use of antimicrobial drugs in the treatment of infectious disease has developed multiple drug resistance. Because of this reason sometimes antibiotics show negative effect on the host including allergic reactions, hypersensitivity and immune-suppression. This startling situation of antibiotic resistance in medically important bacteria, need some new and effective therapeutic agents. Therefore, some alternative antimicrobial drugs from medicinal plants have to be developed for the treatment of infectious diseases[1].

Plant originated antimicrobials have immeasurable remedial potential. They are effective in the treatment of infectious diseases while concurrently extenuating many of the side effects that are repeatedly associated with man-made antimicrobials. The beneficial medicinal effects of plant materials usually result from the combinations of secondary metabolites present in the plant such as tannins, alkaloids, phenol and steroids flavonoids, resins ,steroids, fatty acids and gums which are proficient for producing definite physiological action on body.

The purpose of this work is to evaluate and monitor antibacterial activity of crude methanolic extract and to find out minimum inhibitory concentration (MIC) against *Cinnamomum* extract from gram positive bacteria[2]. In modern time the interest to estimate medicinal plants possessing antibacterial activity for various diseases is on the rise[3].

Cinnamomum zeylanicum

Hindi name-Dalchini Common name- Cinnamon

Cinnamomum zeylanicum bark is commonly used as food additive all over the world. In medicine it is used for cure of cold. It has also been used to cure diarrhoea and other digestive system problems. *Cinnamomum* is high in antioxidant activity[4]. The essential oil of *Cinnamomum* also has antimicrobial properties[5], which is used in the preservation of certain foods. It has been reported to have remarkable pharmacological effects in the treatment of type II diabetes .It has also been used to treat toothache and bad breath.

Bacillus subtilis

Bacillus subtilis is a really diverse bacterial species that is proficient to growth within many environments. It can be isolated from many environments- terrestrial and aquatic, making it omnipresent and broadly adapted to grow in diverse settings within the biosphere However, like all members of the genus Bacillus, B. subtilis can form highly resistant inactive endospores in response to nutrient deficiency and other environmental stresses[6,7]**.** Most strains are non pathogenic. *Bacillus subtilis* may give rise to panophthalmitis conjunctivitis and iridochoroiditis in human.

II. MATERIALS AND METHODS

A. Collection of Samples

Barks of *Cinnamomum zeylanicum* were taken to conduct the protocol. Barks were purchased from the market in Agra (U.P.) from Ayurveda shops.

B. Collection of Bacteria

The bacteria were isolated, characterizes also subjected to standard biochemical tests[8] before the experiments. To obtain pure culture of *B.subtilis* serial streaking of single colony was done on Mueller Hinton agar. Pure cultures of bacteria were maintained in Nutrient broth until further tested.

C. Isolation

1) *Serial dilution Method:* In this procedure a small measured weight is mixed with large volume of sterile water called diluents or dilution blank. Serial dilutions are later prepared by transferring a known volume of the dilution to second dilution blank and so on.
2) *Material Required*
   - *a)* Soil sample
   - *b)* Distilled water
   - *c)* Sterile test tubes
   - *d)* Micro pipette
   - *e)* Micropipette tips
   - *f)* Cotton
   - *g)* Marker
3) *Procedure*
   - *a)* Collected soil samples from a field, mixed thoroughly to make a composite sample for microbiological analysis.
   - *b)* Labeled test tubes 1, 2, 3, 4 (as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) and one unmarked test tube is taken.
   - *c)* 9ml distilled water is added to all of the test tubes
   - *d)* Added 1 gm sample (soil) of finely pulverized, air dried soil into unmarked test tube and vigorously shaked the dilution for 10-15 minutes to obtain uniform suspension of micro organisms.
   - *e)* Kept the above suspension for some time as such to settle down and kept it for 25-30 minutes.
   - *f)* For1:10 dilution (10^{-1}) to the no.1 test tube added 1ml of the solution from the unmarked, kept for settled down.
   - *g)* Transferred 1000 µl of suspension from test tube No.1 into test tube No.2 with a sterile pipette under aseptic conditions to make 1:100(10^{-2}) dilution, shaked it and kept it for about 5 minutes.
   - *h)* Prepared another dilution 1:1000 (10^{-3}) by pipetting 1000 µl of the suspension into test tube No.3, using a fresh sterile pipette and shake it.

D. Preparation of Media

All media were prepared as per standard formulation given in Bacteriology Manual. The dry ingredients were placed in a beaker, suspended in distilled water and then dissolved the medium completely. The prepared medium was dispended into flask and test tube, and finally sterilized by autoclaving at 121°C for 30 minutes. About 15 ml was poured in Petri dishes aseptically. The plates were incubated at 37°C for 24-48 hour for sterility checking.

1) *Material Required*
   - *a)* *Nutrient agar*
   - *b)* *Distilled water*
   - *c)* *Conical flasks*
   - *d)* *Petri plates*
   - *e)* *Bunsen burner*
2) *Instruments*
   - *a)* *Hot plate*

b) *Autoclave*
c) *Laminar air flow*

3) *Procedure*
   a) Put the weighed amount of nutrient agar 1.260 gm in 45ml of distilled water.
   b) Poured the medium into conical flask.
   c) Heated with agitation to dissolve the constituents on hot plate stirrer.
   d) Plugged the flask containing medium.
   e) Sterilized it at 121°C, 15 lbs pressure, for 15 minutes in an autoclave.
   f) Took out the conical flask from autoclave.
   g) Poured the medium into petridishes quickly under aseptic conditions.
   h) Allowed the media to solidify.

E. Preparation of Plant Extract

1) *Collection of Plant material :* The barks were collected some of them were purchased from market. The selected plant materials were washed with clean water and allow to shade dried for about 2-3 weeks. The dried materials were crushed in an electric grinder to coarse powder. The barks were shade dried, powered and were extracted with methanol using a maceration process and with occasional shaking for 3 days. The extract was then filtered, dried at 50 to 60^{o}C and the residue was weighed and percentage yield was calculated and subjected to preliminary phytochemical analysis. The residue was dried and stored in air tight container.

2) *Extraction of Plant materials:* Crude plant extract was prepared by Soxhlet extraction method as following[9]:

About 40 gm of powder material was uniformly packed in to a thimble and run in Soxhlet extractor . It was exhaustible extracted with 200 ml methanol for the period of about 48 hour or 22 cycles or till the solvent in the siphon tube of an extractor become colorless. After that extract was filtered with the help of filter paper and solvent evaporated from extract in Rotary evaporator to get the syrupy consistency. The residue was dried over anhydrous sodium sulphate to remove trace of alcohol. Then extract kept in refrigerator at 4°C and analyzed their physical and chemical property.

3) *Antibiogram:* Disc diffusion method was used to test antibacterial activity of plant extract[10].

4) *Phytochemical analysis :* Extracts was tested for the presence of active principle such as phytosterols, tannins, flavonoids, saponins, alkaloids, glycoside, triterpenoids and proteins. Standard procedures[11] were used.

III. RESULTS

Table – 1: Antibiotic activity of isolated *Bacteria*

Antibiotic	Symbol	*B.subtilis* (Zone of inhibition)
Gentamycin	G	45 mm

Table – 2: Antibiogram of potential of plant extract (Disc Diffusion method) for *B. subtilis* (0.5gm/ml)

Cinnamomum zeylanicum Diameter of growth of inhibition zones (mm) Extracts (0.5gm/ml)	MINIMUM INHIBITORY CONCENTRATION mg/ml					
	B.subtilis					
Methanol	D	D1	D2	D3	Positive control	Negative control
R1	25 mm	18 mm	12 mm	8 mm	45 mm	Nil
R2	20 mm	20 mm	11 mm	10 mm		
R3	14 mm	15 mm	10 mm	11 mm		

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Table – 3: Antibiogram of potential of plant extract (Disc Diffusion method) for *B.subtilis* (0.25gm/ml)

Cinnamomum zeylanicum Diameter of growth of inhibition zones (mm) extracts (0.25gm/ml)	MINIMUM INHIBITORY CONCENTRATION (MBC) mg/ml (mm)					
	B.subtilis					
Methanol	D	D1	D2	D3	Positive control	Negative control
R1	17mm	11mm	10mm	10mm	45mm	Nil
R2	18mm	12mm	9mm	8mm		
R3	17mm	12mm	8mm	10mm		

Table – 4: Antibiogram of potential of plant extract (Disc Diffusion method) for *B.subtilis* (0.125gm/ml)

Cinnamomum zeylanicum Diameter of growth of inhibition zones (mm) extracts (0.125gm/ml)	MINIMUM INHIBITORY CONCENTRATION mg/ml					
	B.subtilis					
Methanol	D	D1	D2	D3	Positive control	Negative control
R1	14mm	12mm	12mm	11mm	45mm	Nil
R2	15mm	12mm	11mm	10mm		
R3	14mm	11mm	11mm	11mm		

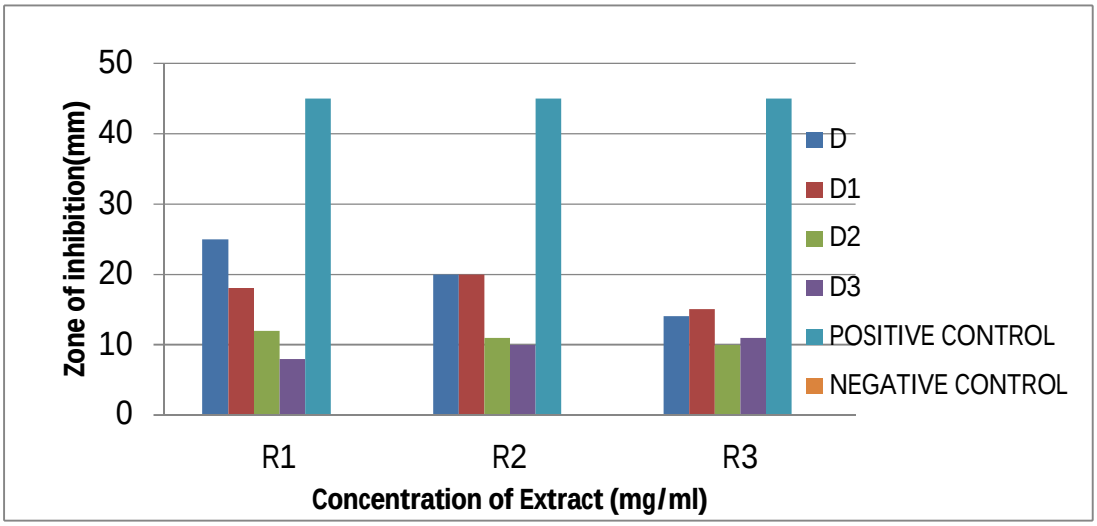

Fig.1: Graphical representation of potential of plant extract against *B. subtilis* (0.5)

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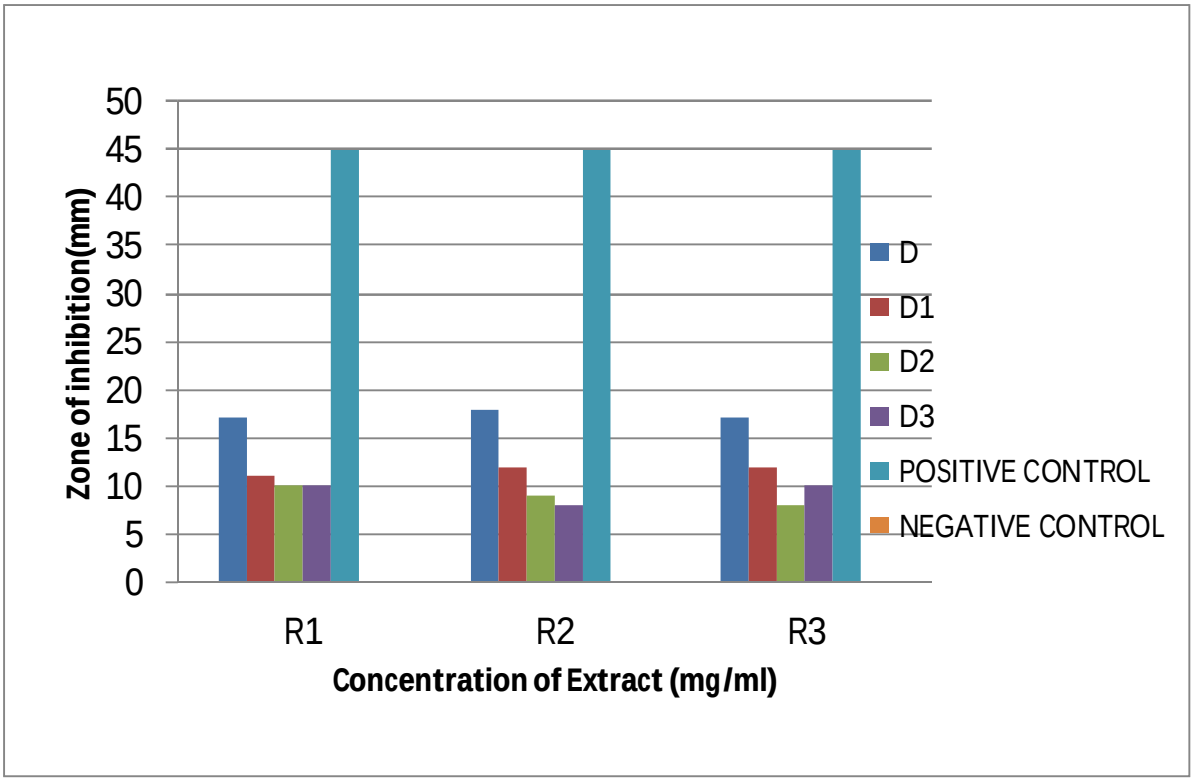

Fig.2: Graphical representation of potential of plant extract against *B. subtilis* (0.25)

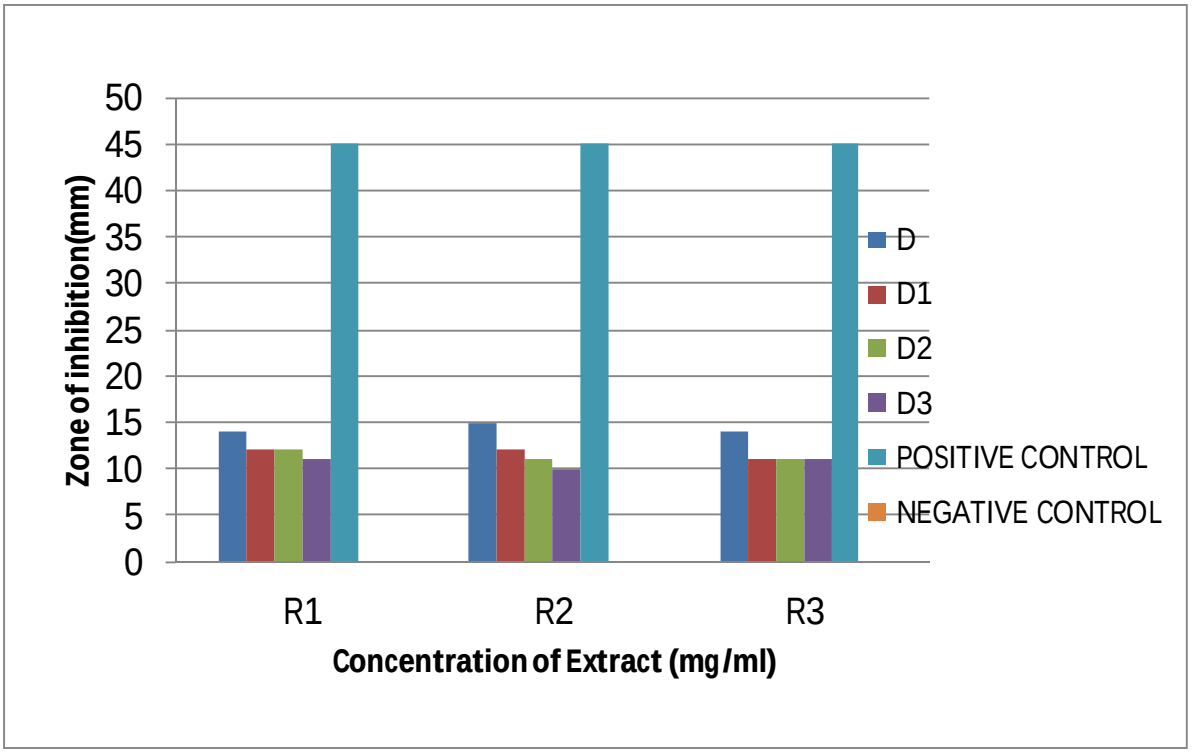

Fig.3: Graphical representation of potential of plant extract against *B. subtilis* (0.125)

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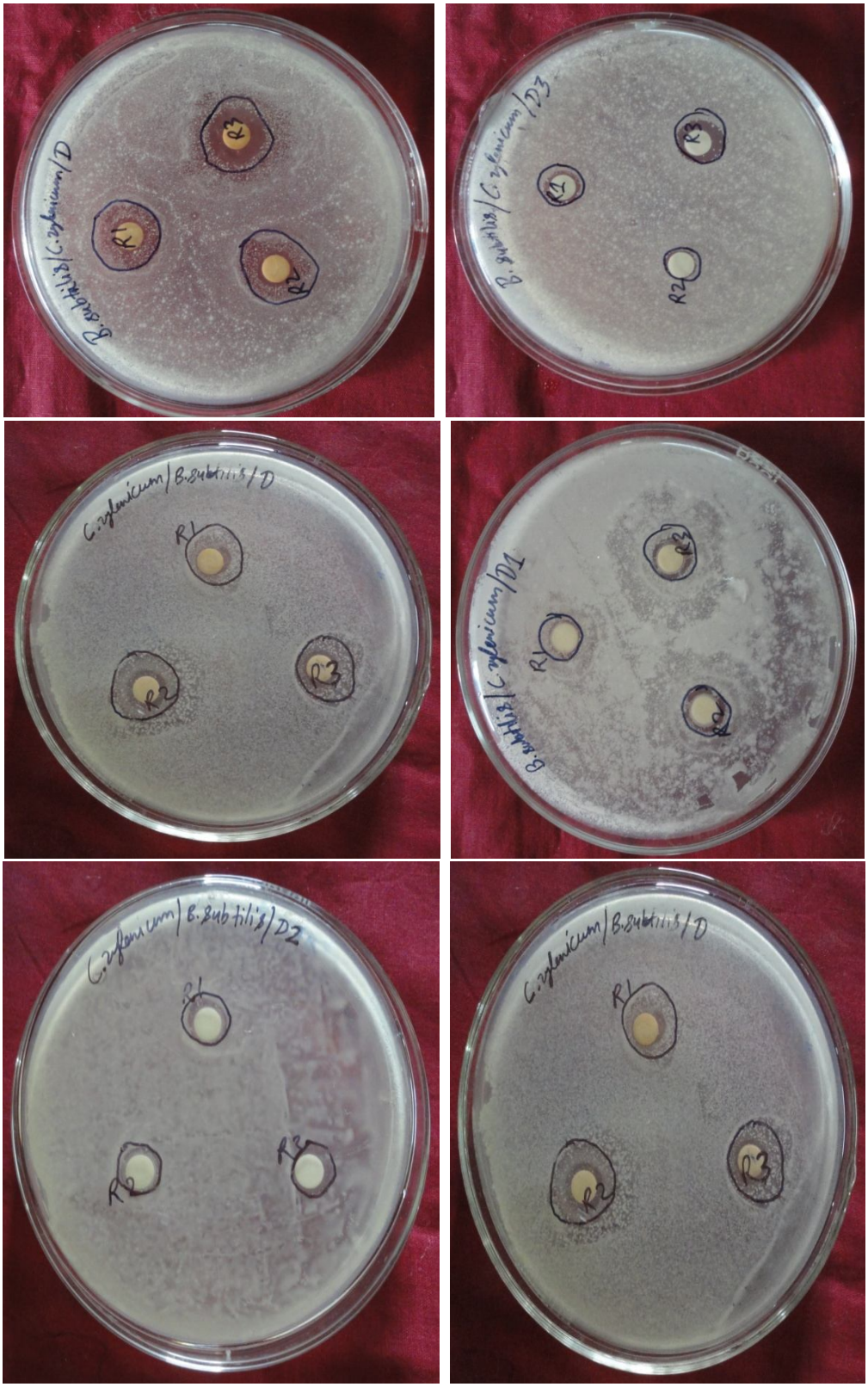

Fig. 4 Antimicrobial Activity of *Cinnamomum zeylanicum* extract against *Bacillus subtilis*

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Table - 5
Antibiogram of Bacteria

Antibiotic	Symbol	B.subtilis (Zone of inhibition)
Panicillin G	P	8mm
Oxacillin	OX	9mm
Erythromycin	E	25mm
Clindomycin	Cd	26mm
Linezolid	Lz	29mm
Co-Trimoxazole	Co	20mm
Vancomycin	Va	19mm
Ciprofloxacin	Cf	20mm
Tetracycline	T	20mm
Cefotaxime	Ce	16mm
Chloramphenicol	C	21mm
Gentamicin	G	35mm

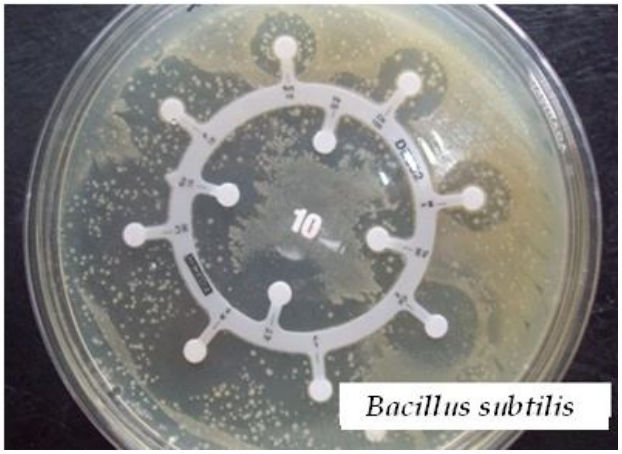


Figure 5: Antibiogram of *B.subtilis*

Table- 6 : Physical property of Cinnamomum zeylanicum extract

S. No.	Extract	Color	Shape	Odor	Taste	Nature	Consistence
1.	Cinnmomum zeylanicum	Pale brown	Compound quill	Fragrant aromatic	Sweet agreable	Sticky	Powder

Table -7: Solubility of extract

S.No	Crude extract	Ethanol	Methanol	D.W.
1.	Cinnamomum zeylanicum	-	+	+

+ = Present, - = Absent

Table-8: Phytochemical analysis of Cinnamomum zeylanicum (Bark) extract

S. No	Crude extract	Alkaloid	Glycoside	Carbohydrate	Protein	Tannin	Flavonoid	Phytosteroid	Saponin	Triterpenoids	Phenols
1.	Cinnamomum zeylanicum (Bark)	+	+	-	-	+	-	+	+	+	+

IV. CONCLUSION

The methanol extract of *Cinnamomum zeylanicum* studied was found to give an antibacterial activity against the pathogenic bacterial strains *Bacillus subtilis.* Methanol extract of *Cinnamomum zeylanicum* gave its maximum size of zone of 25mm in case of *Bacillus subtilis* (0.5gm/ml).

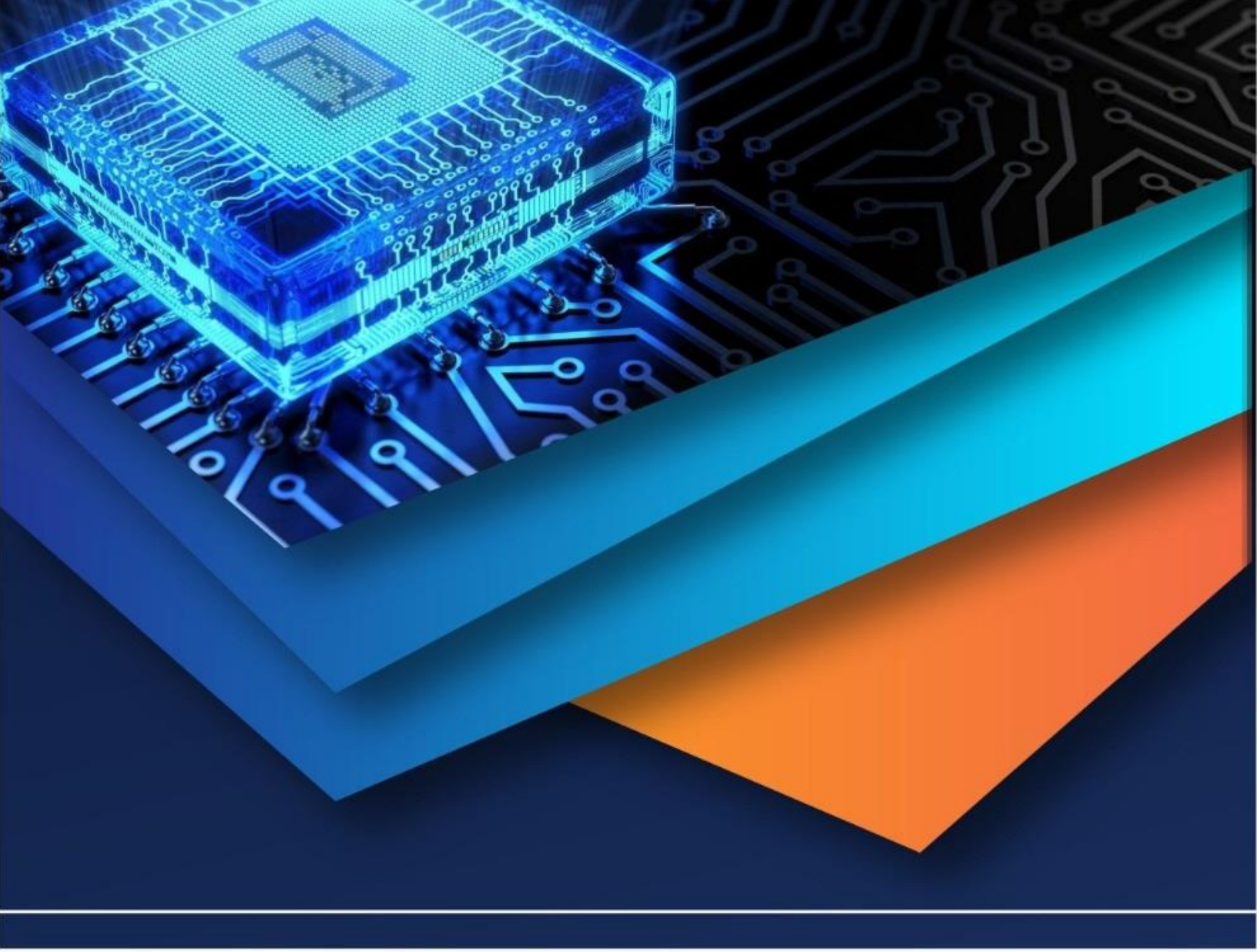
The antibacterial activity has been certified the presence of some phytochemicals in the extracts. Studies recommended that the antibacterial activity of *Cinnamomum* was possibly due to their major component, cinnamaldehyde and their properties could be various. Cinnamaldehyde is a natural antioxidant and the animal studies recommend that an extract of *cinnamon* bark taken orally may help to check stomach ulcer.

An important characteristic of plant extracts and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death[12].

The inhibition produced by the plant extracts against particular organism depends upon various parameters. The antibacterial property may not demonstrate as ZOI due to variable diffusability in agar medium. Therefore MBC value has also been computed in this study. MBC is the lowest concentration of antibacterial substance required to produce a sterile culture[13] **.** This antibacterial study of the plant extracts established that folk medicine can be as effective as modern medicine to fight pathogenic microorganisms.

REFERENCES

[1] Agrawal P., Rai V., Singh R. B. (1996). Randomized, placebo-controlled, single-blind trial of holy basil leaves in patients with noninsulin-dependent diabetes mellitus. International Journal of Clinical Pharmacology and Therapeutics; 34:406-409.
[2] Pokhrel N. R. (2000). Screening and evaluation of the antimicrobial activity of some medicinal plants of Nepal and isolation of pure antimicrobial compound from Bauhinia variegata. A Dissertation Submitted to Central Department of Microbiology, Tribhuwan University, Kathmandu.
[3] Clark, A.M. and Hufford C.D., (2009). Discco and development of novel prototype antibiotics for opportunistic infections related to the acquired immunodeficiency Kathmandu University Journal of Science, Engineering and Technology 5: (I); 143- 150. FAO (1993). Medicinal and Aromatic plant in Asia. Bangkok, Thailand RAPA, Publication.
[4] Mathew S. and Abraham BTE (2006). Food Chemistry; 94: 520–528.
[5] Kamal Raj Aneja, Radhika Joshi and Chetan Sharma (2009). Antimicrobial activity of Dalchini (Cinnamomum zeylanicum bark) extracts on some dental caries pathogens Journal of Pharmacy Research.
[6] Sonenshein, A.L. (2002). Bacillus subtilis and Its Closest Relatives: From Genes to Cells, ASM Press.
[7] Ricca, E. (2004). Bacterial Spore Formers: Probiotics and Emerging Applications, Horizon Scientific Press.
[8] Krieg N R and Holt J G, (1984). Bergey's manual of systematic bacteriology, vol. 1, 4th edition (Baltimore: Williams and Wilkins).
[9] Okeke (2001). Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO, Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity, J Ethnopharmacol; 78: 119 127.
[10] Mukherjee P.K., ,S. N. Giri, K. Saha, M. Pal, and B. P. Saha. 1995b. Antifungal screening of Nelumbo nucifera (Nymphaeaceae) rhizome extract,Indian Journal of Microbiology,35:320-327
[11] Debela, A. (2002). Manual for PhytochemicalScreening of Medicinal Plants. Ethiopian Healthand Nutrition Research Institute, Addis Ababa,Ethiopia, 35-47.
[12] Rastogi, R.P. and Mehrotra B.N. (2002). Glossary of Indian Medicinal Plants. National Institute of science communication, New Delhi, India.
[13] Cheesbrough, M. (1993). Medicinal laboratory manual for tropical countries, ELBS reprinted Edition.



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