International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

Phytochemical Screening and Antibacterial Activities of Cinnamon against Vancomycin Resistant Enterococcus

P. Venkatachalam[1], Jyothiprabha .V[2]

[1]PG & Research Department of Microbiology, Sengunthar Arts and Science College, Thiruchengode-637205, Namakkal, India

Abstract: ***This study is conducted to evaluate the antimicrobial activity of cinnamon (Cinnamomum verum) extracts against Vancomycin Resistant Enterococcus. Different types of organic solvents (Ethanol, Methanol, Acetone, Chloroform and Distilled water) were used to prepare the extracts from cinnamon. The extracts were tested against seven isolates of Vancomycin Resistant Enterococcus. The results showed that methanol, acetone and chloroform extracts exhibited good antimicrobial activity against the bacterial isolates. The extracts were subjected to phytochemical analysis which confirms the presence of glycosides, saponins, phenol and terpanoids. The bioactive components present in cinnamon were also evaluated by GC-MS analysis and the analysis revealed the presence of thirty major compounds. The study concludes that the secondary metabolites present in the spice can be an indispensible source of antimicrobial compounds.***

Keywords: *Cinnamomum verum,* Vancomycin Resistant Enterococcus, GC-MS analysis, antibacterial activity, glycosides

1. Introduction

India is one of the largest producer, consumer and exporter of spices. Spices have been used for not only flavor and aroma of the foods but also to provide antimicrobial properties. Particularly in Ayurveda, spices contributed a major amount for the treatment of key disorders of the body. Homeopathic medicine has been using spices as one of the chief ingredients in most of theirpreparations.Spices are used as substances that increase the taste and variation of food[1], [2]. According to world health organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs [3]. Spices are also used for stabilizes in several food items from deterioration. In addition to these spices are some of the most commonly used natural antimicrobial agents in foods. Some of the natural compound found in various spices possesses antimicrobial [4]. Phytochemicals are bio- active chemicals of plant origin. Medicinal plants are useful for healingas well as for curing of human diseases because of the presence of phytochemical constituents [5].*Cinnamomum verum* commonly known as cinnamon is a spice obtained from the bark of a tree in the family Lauraceae.

2. Methodology

Collectionand Preparation of Cinnamon Extracts

The spice cinnamon was collected from Spices Research Centre Calicut. The spice was cleaned and washed in sterile distilled water and air dried at room temperature. The dried spices was powdered using blender.10 gram of powdered spices were weighed and mixed with 100 ml of five different solvents (methanol, ethanol, acetone, chloroform and distilled water) in conical flasks and kept in rotatory shaker at 150 rpm for 24 hours. After 24 hours it was filtered with Whatman No.1 filter paper. The filtrates were evaporated in a hot air oven at 40°C until dry. One gram dried extracts were resuspended in 10 ml of Dimethyl Sulphoxide (DMSO) individually. The extracts were stored in sample bottles at 4° C prior to use.

Test microorganism

The test organisms used in this study are clinically isolated Vancomycin Resistant Enterococcus. The clinical samples blood, urine and sputum were procured from Perundurai Medical College Hospital, Perundurai. The transported samples were processed within 2-4 hours. The samples were inoculated on Nutrient agar, Blood agar and Bile Esculin Agar. The plates were incubated at 37° C for 24 hours. The colonies are developed in this medium is pure cultured and identified based on their biochemical characteristics given in Bergey's manual of systematic classification.

Antibiotic sensitivity test

Antibiotic sensitivity test by Kirby-Bauer Method(1966/1956). The antibiotics used in this study are Norfloxacin, Amikacin, Ciprofloxacin, Penicillin G, Amoxicillin, Methicillin, Oxacillin, Chloramphenicol, Clindamycin, Erythromycin, Gentamycin, Ampicillin, Kanamycin, Cloxacillin, Nitrofurantoin, Vancomycin, and Pefloxacin.The selected strains of bacteria were inoculated into 2 ml of sterile nutrient broth, and incubated at 37°Cfor 16-18 hours. Using a sterile cotton swab,the cultures were aseptically swabbed on the surface of sterile Mueller-Hinton Agar plates. Using an ethanol dipped and flamed forceps, the antibiotic discs were aseptically placed over the seeded MHA plates. The plates were incubated at 37°C for 24 hours and the diameter of the inhibition zones was measured in mm.

Antibacterial activity testing using agar well method

The selected strains of bacteria were inoculated into 2 ml of sterile nutrient broth, and incubated at 37°C for 16-18 hours. Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile Mueller- Hinton Agar plates and left to dry for few minutes at room temperature. Agar wells were prepared with the help of sterilized cork borer. Different volume of spices extracts (50µl, 100µl, 150µl, 200µl) were added to different wells in the plate. The plates were incubated in upright position at 37°C for 24

hours. The diameter of inhibition zones were measured in mm and the results were recorded.

Phytochemical analysis

The cinnamon extracts were subjected to phytochemical screeningfor the presence of alkaloids, carbohydrates, flavonoids, terpanoids, saponins, phenol, tannins, glycosides, steroids and proteins as per standard methods [6].

GC- MS Analysis

GC-MS technique was used in this study to identify the components present in the cinnamonextract. GC- MS technique was carried out atThe South India Textile Research Association, Coimbatore, TamilNadu, India [SITRA].GC-MS analysis was carried out usingthermo gc - trace ultra ver: 5.0, thermo ms DSQ II system. The Column is db 5 - ms capillary standard non - polar columns(30 mts: id 0.25 mm, film: 0.25 µm). The GC oven temperature was 70^{o}C and raised to 260^{o}C at a rate of 6^{o}C /min. Purified helium was used as a carrier gas at constant flow rate of 1ml/min. The injector and detector temperature was 250^{o} C. Electron impact mass spectra were obtained at 70 eV by operating in full scan acquisition mode in the range of m/z 50-650. The identification of the active compounds were performed by comparing the obtained mass spectra with those from the Wiley and NIST spectral library and their retention time and mass spectral with those of the reference compounds.

3. Results and Discussion

The present study is an important report for presence of phytochemical and antimicrobial action ofcinnamon using various solvent extracts such as Ethanol, methanol, acetone, chloroform and distilled water. The selection of various solvents is based on the difference their polarities. The main aim to choose different solvent is the maximum solubility of different constituents of cinnamon powder will depend upon the polarity of solvents. The objective of the study is to investigate antimicrobial activity against vancomycin resistant enterococcus and also identification of phytochemical compounds for the activity by GC-MS.

Antibacterial activity of cinnamon was investigated against vancomycin resistant enterococcus.Ethanol, methanol, acetone, chloroform and distilled waterextracts of cinnamon were tested for their antimicrobial activity against vancomycin resistant enterococcus isolates. A good response was seen with extracts of cinnamonshowing the maximum inhibitory zone towards all the isolates.Antibacterial test results of cinnamon are shown in table 1. At the end of the analysis Cinnamon showed good antibacterial activity.The highest zone of inhibition of 21mm was recorded with the acetone extracts of cinnamon against all the isolates. Chloroform showed moderate antibacterial activity. Methanol anddistilled water showed least antibacterial activity. Ethanol has no antibacterial activity. The present study support that cinnamon bark has antibacterial activity against Vancomycin Resistant Enterococcus.

Cinnamon was detected to exhibit a similar inhibitory effect against *P.aerogenosa* and *E. faecalis*, and its weakest activity was against *E.coli* and *M luteus*[7]. The eugenol shown to have a stronger bactericidal activity against *E.coli* and *K.pneumoniae*[8]. Previously, cinnamon and clove had a strong inhibitory activity against microorganisms [9].

Phytochemical analysis of cinnamon showed the presence of glycosides, saponin, phenol and terpanoids in all cinnamon extracts.Carbohydrates were present only in acetone cinnamon extract. The tannins, alkaloids, flavonoids, carbohydrates, and steroids were absent in all cinnamon extracts.In the present investigation glycosides are present in cinnamon extracts. The glycosides are useful in lowering blood pressure [10]. They are also used in the treatment of congestive heart failure and cardiac arrhythmia. Terpanoids are also present in the studied spices. Terpanoids are used in the treatment of cough, asthma and hay fever. Saponins are present in cinnamon extracts. Saponins protect against hypercholesterolemia and antibiotics properties [11].

This report is the first of its kind to analysis the chemical constituents responsible for the antimicrobial activity of cinnamon.GC-MS analysis of sample contained 50-75 compounds that matched NIST library data.GC-MS phytochemical screening result of cinnamon extract showed the presence of major thirty compounds.The results were recorded in table-3. The identified bioactive compounds possess many biological properties. The active principle molecular weight(MW), molecular formula (MF) and their retention time (RT) have been detected. Some important antimicrobial compounds reported in cinnamon are (-)-Caryophyllene oxide ,Benzofuran, 2-methyl, Phenol, 2-methoxy-4-(2-propenyl), Tetratetracontane, ë-Cadinene, trans-Caryophyllene, Chlorotribromomethane, Anodendroside F, 1H-Purin-6-amine, 3,5-Diphenyl-2-(3',4'-dimethoxyphenyl)-pyrrole, Cubenol were seen to be present in the extract. Analysis results also showed lot of compounds with area percentage less than 1%.

Table 1: Antimicrobial Activity of Cinnamon Against Vancomycin Resistant *Enterococcus* [VRE]

VRE ISOLATE	*ETHANOL* 50 100 150 200	*METHANOL* 50 100 150 200	*ACETONE* 50 100 150 200	*CHLOROFORM* 50 100 150 200	*DISTILLED WATER* 50 100 150 200
VRE 1	-- -- -- --	-- -- -- 14	-- 17 19 21	-- -- 13 16	-- -- -- --
VRE 2	-- -- -- --	-- -- -- --	-- 16 18 20	-- -- 16 18	-- -- -- 15
VRE 3	-- -- -- --	-- -- -- 13	-- 15 17 19	-- -- 14 16	-- -- -- --
VRE 4	-- -- -- --	-- -- -- --	-- 17 19 20	-- -- 13 15	-- -- -- 13
VRE 5	-- -- -- --	-- -- -- 14	-- 16 18 20	-- -- 13 16	-- -- -- --
VRE 6	-- -- -- --	-- -- -- 15	-- 17 19 21	-- -- 14 18	-- -- -- 15
VRE 7	-- -- -- --	-- -- -- 14	-- 16 18 21	-- -- 15 17	-- -- -- 14

Table 2: Phytochemical Analysis Of Cinnamon Extracts

No	*Phytochemical Test*	*Methanol*	*Ethanol*	*Acetone*	*Chloroform*	*Distilled Water*
1	ALKALOIDS	_	_	_	_	_
2	GLYCOSIDES	+	+	+	+	+
3	SAPONNIN	+	+	+	+	+
4	PHENOL	+	+	+	+	+
5	STEROIDS	_	_	_	_	_
6	FLAVANOIDS	_	_	_	_	_
7	TERPANOIDS	+	+	+	+	+
8	PROTEIN	_	_	_	_	_
9	TANNINS	_	_	_	_	_
10	CARBOHYDRATES	_	_	_	_	_

'+' = present '-' = absent

Table 3: Phytochemical Analysis Of Cinnamon Extract Using GC-MS Analysis

S.NO	*MW*	*R.T*	*Formula*	*Compound Name*	*AREA %*
1	370	16.37	$C_{22}H_{26}O_5$	CALANOLIDE A	0.98
2	132	25.72	C_9H_8O	Benzofuran, 2-methyl- (CAS)	35.41
3	164	17.78	$C_{10}H_{12}O_2$	Phenol, 2-methoxy-4-(2-propenyl)- (CAS)	21.03
4	204	20.33	$C_{15}H_{24}$	trans-Caryophyllene	4.99
5	204	14.40	$C_{15}H_{24}$	à-Humulene (CAS)	1.31
6	204	16.60	$C_{15}H_{24}$	à-Muurolene	3.03
7	204	39.14	$C_{15}H_{24}$	ë-Cadinene (CAS)	3.85
8	301	5.20	$C_{17}H_{19}NO_4$	Papaveroline, 1,2,3,4-tetrahydro-5-O-methyl-	0.27
9	220	67.72	$C_{15}H_{24}O$	(-)-Caryophyllene oxide	1.98
10	355	85.72	$C_{15}H_{13}N_7O_4$	1,3-Dimethyl-2,4-dioxo-6-methyl-8-(p-nitrophenyl)-1,2,3,4-tetrahydro[1.2.4]triazolo[3,4-f]purine	0.24
11	222	21.79	$C_{15}H_{26}O$	Cubenol	0.29
12	355	42.34	$C_{24}H_{21}NO_2$	3,5-Diphenyl-2-(3',4'-dimethoxyphenyl)-pyrrole	1.13
13	222	6.50	$C_{15}H_{26}O$	Farnesol	0.24
14	400	16.29	$C_{27}H_{44}O_2$	2à/2á-Hydroxycholest-4-en-3-one	0.22
15	282	14.32	$C_{17}H_{14}O_4$	2,3-Dimethoxy-6-methylanthraquinone	0.49
16	329	17.21	$C_{19}H_{23}NO_4$	(3R*,4S*)-3-(2-Nitro-4-methoxyphenyl)-4-(4-hydrox yphenyl)hexane	0.62
17	237	20.61	$C_{16}H_{15}NO$	6,7,8,9,10,11-hexahydro-5H-benzo[h]cyclopent[c]is oquinolin-5-one	0.20
18	529	13.41	$C_{23}H_{51}NO_3Si_5$	NOREPINEPHRINE-PENTATMS	0.25
19	498	11.12	$C_{27}H_{54}O_4Si_2$	1-Monolinoleoylglycerol trimethylsilyl ether	0.22
20	382	7.08	$C_{21}H_{34}O_6$	Pregn-5-ene-3,8,11,12,14,20-hexol, (3á,11à,12á,14á,20R)-	1.25
21	243	32.96	$C_{12}H_{10}FN_5$	1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS)	0.57
22	386	27.81	$C_{27}H_{46}O$	Cholesterol	5.35
23	618	7.23	$C_{44}H_{90}$	Tetratetracontane (CAS)	7.37
24	238	6.65	$C_{13}H_{18}O_4$	5-Methyl-2-methylene-10-oxo-3,4,7,8,9,10-hexahyd ro-2H-oxecine-3-carboxylic acid, methyl ester	0.35
25	277	19.21	$C_{14}H_9D_6NO_3S$	N-(2'-hydroxy-5'-methylphenyl)-4-(methylbenzenesu lfon)amide	0.19
26	414	16.40	$C_{30}H_{54}$	Cyclohexane, 1,1',1",1'"-(1,6-hexanediylidene)tetrakis- (CAS)	2.38
27	284	53.45	CBr_3Cl	Chlorotribromomethane	0.59
28	562	16.58	$C_{30}H_{42}O_{10}$	Anodendroside F	1.94
29	610	26.88	$C_{32}H_{34}O_{12}$	Hexamethyl 2,11,13,16-tetramethyltricyclo[10.3.1.0(3,13)]hexade ca-2,4,6,8,10,12(16),14-heptaene-4,5,8,9,14,15-hex acarboxylate	2.88
30	431	9.90	$C_{23}H_{37}NO_3Si_2$	Dihydromorphine , di(trimehylsilyl) ether	0.39

'MW'- Molecular Weight. 'R.T' – Retention Time.

4. Conclusion

The bark of cinnamon extracts possesses a significant inhibitory effect towards the potentially serious vancomycin resistant enterococcus isolates. The study explores the goodness of cinnamon which has a commensable sense of and can be used as a plant of phytopharmaceutical importance. The result of this study would lead to find out some compounds which are very useful for the manufacturing of new drugs.

References

[1] Ceylan A. Medical Plants-ll Volatile Oil Plants. Ege University, Faculty of Agriculture, Department of Field Crops, lzmir, Turkey,1997.
[2] Bulduk S. Food Technology. 2nd edition Daley Publishing Ankara, Turkey , 2004.
[3] Himal Paudel Chhetri, Nisha Shrestha Yogol, Jyoti Sherchan. Anupa. Phytochemical and antibacterial evaluations of some medicinal plants of Nepal, Vol 1, pp 49-54, 2008.
[4] Hatha AAM, Indu MN, Harsha U. Antibacterial activity of some of the south- Indian spices against serotype of

Escheichia coli, Salmonella, Listeria monocytogenes and Aeromonas hydrophilla. Brazili j of microbiol 37:153-158, 2006.

[5] Nostro A, Germano MP, Dangelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl.Microbiol 30: 379-384, 2000.

[6] Chitravadivu C., Manian S.,and Kalaichelvi K.. Qualitative Analysis of Selected Medicinal Plants. Tamilnadu. India Midd-East j of Scienti Resea. 4 (3): 144-146, 2009.

[7] Mabrouk SS, El- ShayebNMA. Inhibition of aflatoxin formation by some spices. LebensmUntersForsh 171: 344-347, 1980.

[8] Ramadan FM, El-Zanfaly HT, El- Wakeil FA, Alian AM.On the antibacterial effects of some essential oils.I. Use of agar diffusion method.ChemMikrobiolTechnol.Lebensm 25: 51-55,1972.

[9] Barbosa Canovas GV, Pothakamury VR, Paulo E, Swanson BG. Non-thermal preservation of foods. Marcel Dekker Inc New York , 1998.

[10] Nyarko A. A., Addy M. E.. Effects of aqueous extract of *Adenia cissampeloides* on blood pressure and serum analyze of hypersensitive patients. Phytotherapy Res. 4(1): 25-28, 1990.

[11] Amin Mir M., Sawhney, S.S. and Jassal, M.M.S. Qualitative and quanditative analysis of phytochemicals of *Taraxacumofficinale*.Wudpecker J. Phar. and Pharmaco. 2 (1): 001-005, 2013.