Octa Journal of Biosciences
International peer-reviewed journal
Octa. J. Biosci. Vol. 2(1):49-52

ISSN 2321 – 3663
June 2014

Octa Journal of Biosciences

journal homepage: www.sciencebeingjournal.com

Antimicrobial activity of plants (*Cinnamomum zeylanicum, Cedrus deodara, Eucalyptus globulus, Rosmarinus officinalis*) essential oils against some bacterial and fungal strains

Afshan Tarranum*, U.R. Malhotra, A. Ghildiyal and P. Chandola

Dept. of Lifescience, Beehive College of Advance Studies, Dehradun, U.K., India

ARTICLE INFO

Received 12 March. 2014
Revised 7 May 2014
Accepted 30 May 2014
Available online 16 Jun. 2014

Keywords: Agar disc diffusion; Antibacterial; Essential oils; MBC.

Email:afshantarannum2003@yahoo.co.in

ABSTRACT

The present investigation was done to evaluate the activity of essential oils of (Cinnamomum zeylanicum, Cedrus deodara, Eucalyptus globulus, Rosmarinus officinalis) against different pathogenic bacterial and fungal strains. Different antibiotics were also included as a positive control against bacterial strains used. Cinnamomum zeylanicum was found effective against all the bacterial strains used and one of the fungi used. Cedrus deodara showed moderate antibacterial activity against six of the strains used. Eucalyptus globulus also showed moderate inhibitory activity against seven of the bacterial strains used. Rosmarinus officinalis showed selective inhibitory activity against some of the bacterial strains used. The oil of Cinnamomum zeylanicum showed moderate activity against Candida albicans. Tetracycline and Ofloxacin were tested against the bacterial strains used. Ofloxacin was found to be more effective as compared to tetracycline. Cinnamomum zeylanicum was found to be more active so we performed its Minimum Bactericidal Concentration which showed Cinnamon oil to be active against different bacterial strains. The results showed that oil of Cinnamomum zeylanicum was far more effective in controlling the growth of bacterial strains in all the oil used. It was found to be more effective than the antibiotics used.

INTRODUCTION

Essential oils are complex volatile compounds produced in different plant parts, which are known to have various functions in plants including conferring pest and disease resistance. The complexity in essential oils is due to terpene hydrocarbons as well as their oxygenated derivatives, such as alcohols, aldehydes, ketones, acids and esters (Tzortzakis et al., 2007).

Cinnamomum zeylanicum is one of the oldest herbal medicines known, having been mentioned in Chinese texts as long as 4,000 years ago. It is often used for medicinal purposes due to its unique properties. It has been established that the oils and extracts from cinnamon possess a distinct antioxidant activity, which is especially attributed to the presence of phenolic and polyphenolic substances (Jayaprakasha et al., 2002; Tomaino et al., 2005).

The evolution and biogeography of Cedrus deodara are great interest to botanists. It is an ever-green tree with spreading horizontal branches having small leaves (Parveen R et al., 2010). Cedrus (true cedars) a very important genus of trees belonging to Pinaceae, has a selected distribution in the Mediterranean region. Some work has been done on Cedrus deodara oil but on different line of assessment such as enzyme activities in the nervous tissue of terrestrial snails, cell pathology, prevalence of allergic response by pollen allergens, cytotoxic activity during in vitro screening of medicinal plant extracts (Rao et al., 2003; Bist et al., 2005; Shashi et al., 2006; Nisha et al., 2007).

Eucalyptus is one of the world's important and most widely planted genera. It is a tall, evergreen tree, native to Australia and Tasmania, successfully introduced worldwide, now extensively planted in many other countries. It has been shown that essential oil of Eucalyptus globulus Myrtaceae (Tasmanian Blue Gum) grown in Montenegro possesses rather a significant activity against different microorganisms, including human pathogens, food poisoning and spoilage bacteria, and blastomycete opportunistic fungus C. albicans. These results confirm the potential use of E. globulus essential oil in the food and pharmaceutical industries, which may be useful as an alternative antimicrobial agent in natural medicine for the treatment of numerous infectious diseases. (Delaquis et al., 2002).

Rosmarinus offincinalis is an aromatic plant and thus a flavouring agent, widely used in foods. Its extracts have been introduced as preservatives in the food industry. Rosemary extract formulations are the only ones commercially available for use as antioxidants in the European Union and the United States, and they are marketed in an oil-soluble form, as a dry powder, and in water-dispersible or water-miscible formulations. The non-nutrient secondary metabolites of rosemary such as the phenolic diterpenes, carnosol, carnosic acid, methyl carno-

-sate, rosmanol, and epirosmanol, and phenolic acids such as ferulic, rosmarinic, and chlorogenic and caffeic acids, have already been reported to possess diverse biological activities, including antioxidant and antimicrobial activity. (Klancnik A et al.,2009).

Although the antibacterial activities of the essential oils from many herb species have been extensively surveyed (Rios & Recio, 2005), their antimicrobial mechanisms have not been reported in great details. Any individual essential oil contains complex mixtures of such compounds, however, little is known about the effect of the interaction between the individual constituents on the antimicrobial activity. Interactions between the constituents may lead to additive, synergistic, or antagonistic effects (Delaquis et al., 2002).

Therefore, the following study was done with the following objectives:

(I) Screening of the essential oils against pathogenic bacteria and fungi.

(ii) Screening for bactericidal activity of the most active essential oil.

MATERIALS & METHODS

Collection of essential oils of plants

The essential oil of (Cinnamomum zeylanicum, Cedrus deodara, Eucalyptus globulus, Rosmarinus officinalis) were collected from local area of Dehradun. The bacterial culture (S.aureus, S.mutans MTCC497, S.gardoni MTCC2695, Staphylococcus, P.mirabilis MTCC425, P.vulgaris MTCC426, Listeria monocytogenes MTCC657, Clostridium perfringens MTCC450) and fungus cultures (Aspergillus niger MTCC281, Candida albicans MTCC183) required for the experiment was procured from Institute of Microbial Technology, Chandigarh.

Antimicrobial Assay

Antimicrobial efficacy of essential oils was evaluated through disc diffusion assay (Lorian V., 1996). DMSO was used as a negative control. The Nutrient Agar Medium (NAM) was prepared and transferred to the sterilized petridishes in such a way to keep a uniform depth of approximately 4mm. The100µl bacterial culture then spreaded over the media surface using a clean sterilized glass rod. Discs were then placed on the centre of agar plates and incubated at 37°C for 2 days. All operations were carried out in laminar chamber in triplicate. Antibiotics (Tetracycline & Ofloxacin) were used as positive control so as to compare the activity of essential oil to antibiotics.

Minimum bactericidal assay

Titreplate Resazurin Assay: The titreplate resazurin assay was performed in 96-well plates. Three-fold dilutions of each bacterial strains and plant were prepared in the test wells in complete nutrient broth, the Twenty microlitres of each bacterial suspension was added to 180 µl of three fold dilution of oil containing culture medium. Control wells were prepared with culture medium and bacterial suspension only. The plates were sealed and incubated for 12 hr at 37°C. After each incubation time, 5µl of resazurin solution were added per well, colouring them blue. Plates were incubated at 37°C for additional 5 hr. After every one hour incubation time intervals plates were read for colour change from blue to pink and pink to colourless in live-bacterial strains containing wells. Oil that showed preliminary microtitre-plate assay were revealed the fast decolouration of resazurin which extracts does not have possessed antibacterial potential. The bioactivity of the extracts were screened by which are all the extracts inhibit the dye reduction (Karuppusamy S et al., 2009).

RESULTS & DISCUSSION

The antimicrobial activity of essential oils is shown in Table 1.1. Results showed that the oil of Cinnamomum zeylanicum was found active almost for all bacterial strains and one of fungal strains (Candida albicans).

Table 1: Antimicrobial activity of essential oils against Bacterial and Fungal Strains.

Bacteria And Fungi	OILS				Antibiotics	
	Cinnamomum zeylanicum	***Cedrus deodara***	***Eucalyptus globulus***	***Rosmarinus officinalis***	**Tetracycline**	**Ofloxacin**
S. aureus	20	3	0	5	0	12
S. gordonii	19	0	0	3	6	15
S. mutans	0	10	2	17	5	18
Staphylococcus sp.	23	14	10	0	0	17
P. mirabilis	21	17	0	6	2	12
P. vulgaris	26	6	0	7	2	0
L. monocytogenes	20	5	9	10	8	13
C. perfringens	0	0	5	0	12	15
C. albicans	21	0	0	0	-	-
A. niger	0	0	0	0	-	-

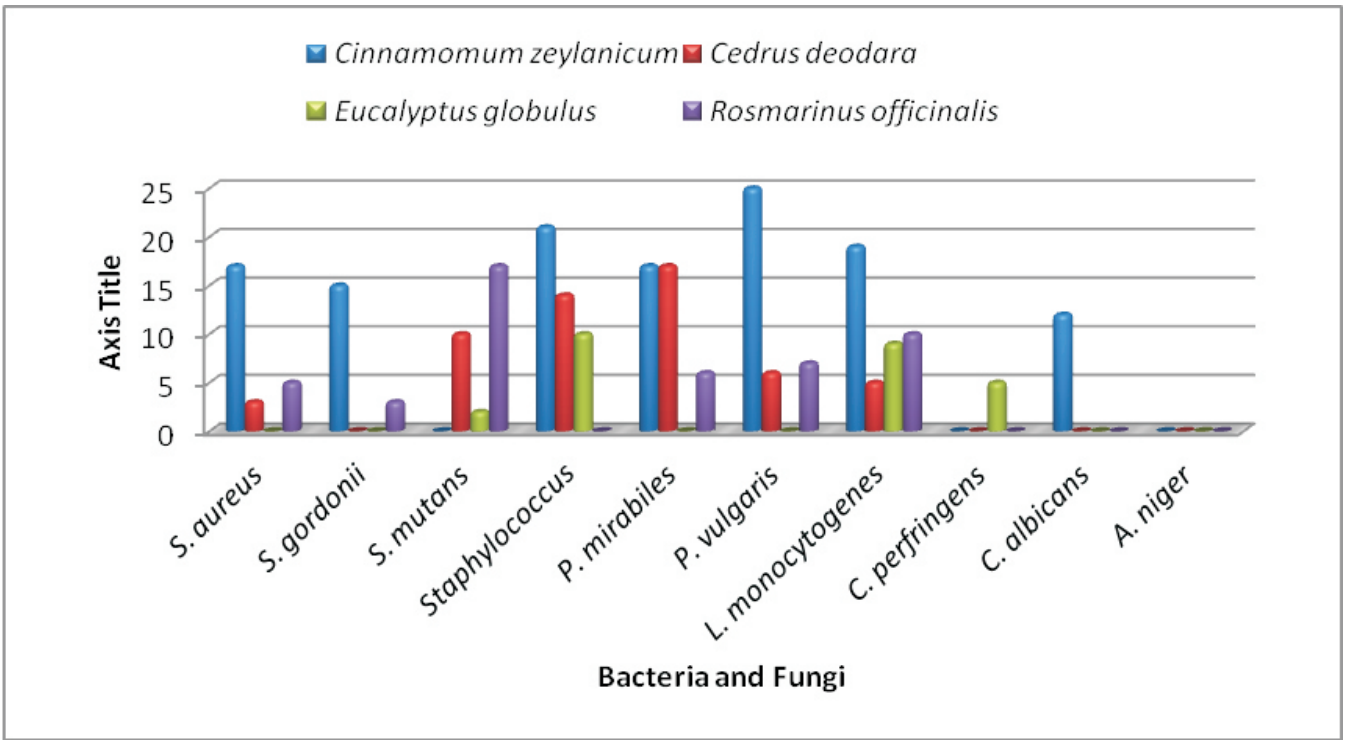

Fig. 1. Comparative activity of different essential oils against different bacteria and fungi

Table 2: Antibacterial screening of Cinnamomum zeylanicum oil from microtitreplate resazurin reduction assay

Bacterial Strains Used	Bacterial Conc.(in µl)								
	C1			C2			C3		
	33%			13.33%			3.33%		
	Oil Conc. (in µl)								
	5 µl	10µl	15µl	5 µl	10µl	15µl	5 µl	10µl	15µl
S.aureus	-	+	+++	++	+	+++	-	-	-
S.mutans	-	+	+++	-	+	+++	-	-	+
S.gordonii	+	-	+	+	+	+	+	+	+
Staphylococcus sp.	+	+	++	+	+	+	+	+	+
P.mirabilis	+	+	+++	+	+	+	+	-	-
P.vulgaris	+	+++	+++	+	+	+++	--	-	-
L.monocytogenes	+	+++	+++	-	-	+++	--	-	-
C.perfringens	+	+	+	-	+++	+++	-	-	+++

+ indicates positive antibacterial activity - indicates negative antibacterial activity

The study evaluated the recently discovered resarzurin dye reduction method for rapid screening of oil for its antibacterial potential. Results were obtained in a short period of time and with very good sensitivity. As is evident from the above table that oil was active against most of the bacterial strains tested at the highest concentration that is 15µL showing persistence of blue colour after the incubation time. This indicates bactericidal activity of oil against the bacterial strains tested.

The antimicrobial activity of essential oil tested against antibiotics shows that the Cinnamon oil is more reactive to bacterial strains as compare to antibiotics which is given in the Table 2.

Among different bacterial and fungal strains Cinnamon oil showed maximum inhibitory effect (ZOI=26mm) against P. vulgaris and minimum inhibitory effect (ZOI=19mm)against S.godonii. This agreed with the result reported by Fani M M et al., 2011 that in vitro studies Cinnamon oil exhibited inhibitory activity on all the microorganisms tested but their effectiveness varied. The results obtained concluded that the bacterial species tested against the essential oil of Cinnamon were inhibited to a considerable extent. This has been supported with the review by R Priyanga et al,2013 in which the antimicrobial

potential of Cinnamomum zeylanicum has been evidenced with several papers and studies published in wide variety of journals. It has been shown by Dubey R C et al in 2005 that CZ essential oils inhibited growth of all organisms including Salmonella typhi, Staphylococcus *aureus, Escherichia coli, Klebsiella pneumoniae* and *Bacillus* subtilis which is in agreement with our studies. It has also been shown by Khan R et al in 2009 that Multi drug resistant (MDR) strains of *Escherichia coli, Klebsiella pneumoniae and Candida albicans* were sensitive to antimicrobial activity of *Cinnamomum zeylanicum* oil. Such high activity against a wide variety of microorganisms opens up new avenues of research in the field of search of new antimicrobial compounds.It was also observed that oil of *Cinnamomum zeylanicum* was more reactive than antibiotics tested against some bacterial strains.

CONCLUSION

It was observed by comparitive evaluation of activity of different oils used that oil of Cinnamomum zeylanicum was most active against the baterial strains used. Furthermore, it showed promising bactericidal activity against some bacterial strains. Therefore, oil of Cinnamomum zeylanicum presents a good scope to be developed as a potential antibacterial agent in suitable formulation with least amount of side effects.

REFERENCES

1. Bist.A., I. Kumar, I. Roy, P. Ravindran, S.N. Gaurs and A.B. Singh (2005). Clinico-immunologic evaluation of allergy in Himalayan tree-Pollen in a topic subjects in India -- a new record. Asian. Pae. *J. Allergy, Immunol*. 23(2-3): 69-78.

2. Delaquis.P.J., Stanich K., Girard B., Mazza G. (2002) . Antimicrobial activity of individual and mixed fractions of dill, cilanto, coriander and Eucalyptus essential oils. *International Journal of Food Microbiology*. 74:101–109.

3. Dubey R.C., Rana A, Shukla R.K. (2005): Antibacterial activity of essential oils of some medicinal plants against certain human pathogens. *Indian Drugs*. 42: 443-446

4. Fani .M.M., Kohanteb J(2011). Inhibitory Activity of Cinnamomum zeylanicum and Eucalyptus globulus Oils on Streptococcus mutans, Staphylococcus aureus, and Candida species Isolated from Patients with Oral Infections. *Shiraz Univ Dent J.*11:14-22.

5. Jayaprakasha.G.K., Rao J, Sakariah KK (2002).Chemical composition of volatile oil from Cinnamomum zeylanicum buds. *Z Naturforsch* (C). 57: 990-993.

6. Karuppusamy.S. and K.M. Rajasekaran (2009). High Throughput Antibacterial Screening of Plant Extracts by Resazurin Redox with Special Reference to Medicinal Plants of Western Ghats. *Global Journal of Pharmacology* 3 (2): 63-68.

7. Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali S.M., Siddiqui M, Khan A. U. (2009): Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. *Molecules* 14:586–597.

8. Klancnik.A., Bernarda Guzej, Majda Hadolin Kolar, Helena Abramovic , And Sonja Smole Moina (2009). In Vitro Antimicrobial and Antioxidant Activity of Commercial Rosemary Extract Formulations. *Journal of Food Protection*. 72(8): 1744–1752.

9. Lorian V,(1996) Antibiotics in Laboratory Medicine, fourth ed. Williams and Wilkins, Baltimore. *Indust. Res.,* 62: 623.

10. Nisha.M., M. Kalyan Asundarana, K.P. Paily, V. Abidha, P. Vanamail and K. Balaraman. (2007). In vitro Screening of medicinal plant extracts of macrofilaricidal activity. Parasitol. *Res*.100(3):575-579.

11. Parveen, R., Ahmed Azmi, M., Tariq, R.M., Mahmood, S.M., Hijazi, M., Mahmud, S. and Naqvi, S.N.H. (2010).Determination of antifungal activity of Cedrus deodara root oil and its compounds against Candida albicans and Aspergillus fumigatus. *Pak. J. Bot.* 42(5): 3645-3649.

12 Ranasinge P, Shehani Pigera, GA Sirimal Premakumara, , Priyadarshani Galappaththy, Godwin R Constantine and Prasad Katulanda(2013). Medicinal properties of 'true' cinnamon (Cinnamomum zeylanicum): a systematic review. *BMC Complimentary and Alternative Medicine* 13(275) : 1-10.

13. Rao. I.G., A. Singh, V.K.Singh and D.K. Singh (2003). Effect of single and binary combinations of plant-derived molluscides on different enzyme activites in the nervous tissues of Achatina fulica. *J. Appl. Toxicol.*, 23(1): 19-22.

14. Rios.J.L.and Recio M.C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology*. 100: 80–84.

15. Shashi,.B.S., Jaswant, R.J., Madhusudana, S.A., Kumar and Nabi, Q.G. (2006). A Noval lignan composition from Cedrus deodara, induces apoptosis and early nitric oxide generation and human leukemia Molt-4 and HL-60 cells. *Nitric oxide*.14(1):72-78.

16. Tomaino .A., Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pusquale A, SaihaA (2005). Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. *Food Chem*. 89: 549-554.