Comparision of Biological Activities of essential oil of three Gymnosperms against *Salmonella typhimurium*

¹Ravikant Singh, ²Ashutosh Pathak, ³Anupam Dikshit and ⁴Rohit K. Mishra ¹Research Scholar, ²Assitant Professor, ³Professor and ⁴Associate Professor ¹Dept. of Biotechnology, Swami Vivekanand University, Sagar-470228, M.P. India

Abstract

The present investigation were focused on the comparision of biological activities of the essential oil of the three gymnospermous foliages *i.e.*, *Pinus roxburghii* Sarg., *Taxodium distichum* L., and *Thuja occidentalis* L., against *Salmonella typhimurium* (MTCC- 3231). The oils were extracted from the needles and foliages of aforementioned plant species using hydro-distillation method. The antibacterial activity of the extracted essential oils was evaluated against *Salmonella typhimurium* (MTCC- 3231) using broth micro-dilution method recommended by Clinical Laboratory Standards Institute (CLSI). The Inhibition Concentration *i.e.* IC₅₀ and Minimum Inhibition concentrations (MIC) using SpectramaxPlus³⁸⁴, of Molecular Devices Corporation, USA were recorded while Streptomycin as standard was taken. The IC₅₀ value of *P. roxburghii, T. distichum* and *T. occidentalis* were showed 0.788, 0.064 and 1.135 mg/ml respectively. The *T. distichum* was found most effective with their MIC 0.353 mg/ml while *T. occidentalis* found least effective with their MIC 1.629 mg/ml against *S. typhimurium*. Hence, essential oil from foliages and needles of gymnosperms exhibit great potential for the development of eco-friendly, non-toxic, cost effective anti-bacterial formulations after undergoing detailed investigation which is in progress.

Key words: Gymnosperms, Essential oil, Biological activity, Broth Micro-dilution, etc.

Introduction:

P. roxburghii Sarg. (known as Chir pine; family Pinaceae) native of Himalayas and distributed throughout India, Nepal, Bhutan and Pakistan. It is widely distributed from foothill to middle Himalayan region of India. *P. roxburghii* is a large tree attaining up to 28-55 m in height with a trunk diameter reaching up to 2 m (figure 1a). The cones of *P. roxburghii* are ovoid, conic and usually open up to 20 cm to release the seeds (1). *P. roxburghii* oil has been traditionally used to treat cuts, wounds, boils and blisters (2). In addition, phytochemical screening of Pinus needles and stems have pound abundant amounts of vitamin C, tannins, and alkaloids while the stem has been primarily used as a source of turpentine oil (3-5). Some microbiological research suggests that the essential oil on *P. roxburghii* has shown significant anti-fungal activity (6) while alcoholic extract of the needle, stem, and cones are reported to exhibit strong anti-bacterial activity.



Fig1. (a) *P. roxburghii* (b) *T. distichum* (c) *T. occidentalis Taxodium distichum.* (L.) L. C. Rich. (Taxodiaceae), commonly referred to as bald cypress, is an unusual and interesting tree, often growing over 25 m in height and over 300 cm in diameter (figure 1b). The leaves are small, 5–20 mm long, green to yellow-green and appearing two-ranked. Young trees have a pyramid shape but eventually form an irregular flattened canopy. The fruits are cones and are composed of scales forming a woody, brown sphere with rough surface 1.5 to 4 cm in diameter. *Taxodium distichum* (L.) has three extant taxa ranging from the eastern United States through Mexico to Guatemala (7). The trees are used for their wood because heartwood is extremely rot and termite resistant (8). Leaves and cones are rich in essential oils and used traditionally to treat gastro-intestinal, skin, respiratory, inflammation, and infections (9, 10). Flavonoids and diterpenoids are the main secondary metabolites (11). *T. distichum* trees can grow on rivers, lake margins, swamps, wet poorly drained habitats and are tolerant to various soil conditions and air pollution (12). These longlived conifers have been widely used for landscape in many countries. The heartwood of bald cypress is used for building materials, and has been reported to resist the attacks of the subterranean termite (13).

Thuja occidentalis L. (Known as White Cedar; family Cupressaceae); native to Eastern Canada and other regions on United State; widely cultivated as an ornamental plant (figure 1c). *T. occidentalis* has been used to treat bronchial catarrh, psoriasis, rheumatism and uterine carcinomas (14). The essential oil of the plant has been used for disinfectants, insecticides, room sprays, and soft soaps. Cedar leaf oil can be obtained by steam distillation or hydro-distillation of the foliage and is used for the production of perfumes, insecticides, soaps and deodorants (15, 16). The essential oil is an active ingredient in the production of cough suppressants, perfumes and soaps, while many cultivars are grown for ornamental purposes (17).

Material and method:

Extraction of essential oil - The plant materials of *P. roxburghii* Sarg., *T. distichum* L., and *T. occidentalis* L., were collected from Roxburgh Garden, Department of Botany, University of Allahabad, in the month of January. Plant were identified at Department of Botany, University of Allahabad. Leaves (needles, foliages) and branchlets were crushed and hydrolyzed using a Clevenger type Apparatus for 4-5 hours (figure 2a). Essential oils of *T. distichum* (bald cypress) appears as dark yellow, *T. occidentalis* (white Cedar) as yellow in colour followed by

P. roxburghii (chir pine) i.e., pale yellow (figure 2b). Oil content was stored at 4°C until analysis (18).

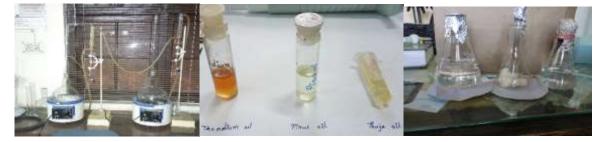


Fig.2a. Clevenger type apparatus. Fig.2b. Extracted oils Fig.2c. DDW and saline media.

Preparation of 0.5 McFarland solution and saline media – Standard method was slightly modified for our study. Dissolve 2.04 ml of H_2SO_4 in 197.69 ml of double distilled water (DDW) (figure 2c). Now add 1% BaCl₂ to the freshly prepared solution (19). 0.5 McFarland solution is ready. Now take the O.D. of the solution. O.D. becomes 0.11, which is within the range of O.D. for McFarland solution. To prepare saline media, dissolve 1 gm of NaCl into 100 ml of DDW. Take the O.D. of this saline media.

Preparation of Mueller-Hinton broth (MHB) – Take 500 ml of DDW in a beaker. Add 10.5 gms of MHB powder. Shake well and boil up to 100 °C. Close the mouth with cotton plug. Place the solution inside autoclave. After this, MHB is ready to use.

Preparation of inocula – 750 μ L of saline media was taken in a culture tube. Add 250 μ L of bacteria in the same tube. Final volume in the culture tube is 1000 μ L. Now take 500 μ L of this solution and add it into another culture tube containing 19.5 ml of MHB, so that the final volume becomes 20 ml. Now inocula will be ready for use.

Antibacterial Screening - Essential oils were screened for antibacterial activity against *S. typhimurium*. Minimum Inhibitory Concentrations (MIC) were determined using Broth Micro-dilution method recommended by Clinical Laboratory Standard Institute (CLSI). 96 well plate was used for microdilutions (figure 3a and 3b). Column-1 contains 190 μ L and 10 μ L of formaldehyde (added after the completion of microdilution). Column-2 contains 200 μ L of MHB. Column-3 contains 180 μ L broth and 20 μ L drug in each row. Row A and B of column-3 contains 20 μ L of streptomycin. Row C and D contains 20 μ L of *Pinus* oil. Row E and F contains *Taxodium* oil whereas row G and H contains *Thuja* oil. Column-3 is known as column of drug control. 100 μ L of broth were added from column-4 to column-12. In column-4, add 80 μ L and 20 μ L drugs in each row one by one as described previously. Now dilute the drugs horizontally from column-4 to column-11. Final volume of each well were 200 μ L. The extract solutions over horizontally diluted 1:1 in MHB in a 96 well plates were incubated at 37 °C for 24 hours (20). The final minimum inhibitory concentration and it was determined as the lowest concentration without turbidity. Streptomycin used as positive control. Formaldehyde was used as a negative control.



Fig.3a. Performing microdilution. Fig.3

Fig.3b. 96 well-plate used for antimicrobial screening

Results:

Percent yield: % yield = weight of oil / weight of sample x 100.

P. roxburghii = 0.110 %, T. distichum = 0.280% and T. occidentalis = 0.40 % (Figure 4). 0.42 0.42 0.400 0.35 0.35 0 280 0.20 0.20 0.21 0.21 0.14 0.14 0.110 0.07 0.07 O 0 coldentalia occidentalis Fig.4. Percentage yield of essential oils. and the second of

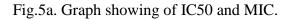


Fig. 5(b). IC50 and MIC values.

The results were recorded in terms oil Inhibition Concentrations (IC50) and Minimum Inhibition Concentrations (MICs) via SpectramaxPlus384, Molecular Devices Corporation, USA. IC50 value of *P. roxburghii*, *T. distichum* and *T. occidentalis* were showed 0.788, 0.064 and 1.135 mg/ml respectively (Figure 5a and b). The minimum inhibition concentrations (MIC) of *P. roxburghii*, *T. distichum* and *Thuja occidentalis* were recorded 1.409, 0.353 and 1.629 mg/ml respectively (Figure 5a and b). *T. distichum* was found to be most effective with their MIC 0.353 mg/m1 whereas *T. occidentalis* was found to be least effective with their MIC 1.135 mg/m1 against *S. typhimurium* (Figure 6a,6b,6c and 6d).

2.500 A4 84	-0.02*	43.019	Sit Day	CV/M	Winhibthon								
			110 005	20.0		- III a	UNEW2			(111+) (#***) (#*	and the second	1999	
	-0.0ma		111111	1111	102 534					ACCOUNTS OF A DESCRIPTION OF A DESCRIPTI	ALC: NO DE CONTRACTOR	WALL NO	
1.250 A5	0.016	40.022	0.009	100.01	102:778	Grange#2 (ang and)							
	0 0/28	HILL STREET	111111	1111		Sample			Matues	Manan Watsins	Titul Core	Children I.	winkikiten
0.025 AB	0.246	0 70	diam'r	1441		The second	2.600	64		0.020	D DOM		1/12 000
EC.	0.111	I I I I I I I I I I I I I I I I I I I	11111	1000		and in the	1325	0-4			and the second second		104,22
0.213 AT		111111111	111111	1111		Contraction of the	1			10.1088	0.008	39.7	78.27
아이어머리에서		11114442	10044	199.0		96.3	10.0074		0.174	In the second second	Linesee-		00.025
		111111111	113111	1159		a high and	I DESCRIPTION OF	Det		1111-11 B 3-41	0.122	36.8	65 172
		0.291	80,450	47.8			41.274.8	5.0	0.402	A 444	11 (3.5.5		214.040
		1111111111	11111	10.2.2	20.773	01.0	all realized to be a second	127			and the local		24.572
	George (1999)	C:390	4.331	0.0	07.801	010	C. COM	CB		D.440	0 414-	20.0	13.104
		111111111	11114	11.2	96.662	64.20	60 CT	200			1.1.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2		48.262
11/11/14/11/14/14		0.0412	6:040	5.4	4.27A	100.00		0.00		C) Deb-g	10.122.2		5,166
		11133414	11111		10.222	81.7	0.030			10.020	0.000		3 0 13
		2.518	4.007	72	94340	and and			0.002				1.846
100000000000000000000000000000000000000	0.0441		10000		an instance of		H.020.	1.7.7	0.204	A DECK OF THE OWNER	A. Canet	-	47.544
	86	0.025 ac 0.024 0.313 eC 0.10 0.400 er 0.144 0.100 er 0.200 840 0.200 840 0.401 950 0.054 950 0.054 840 0.054 840 0.054 840 0.054	0.025 a0 0.024 0.079 0.313 a7 0.514 0.322 0.313 a7 0.514 0.322 0.195 a0 0.260 0.251 0.07% a0 0.565 0.251 0.07% a0 0.565 0.351 0.056 0.565 0.315 0.010 0.470	05 0.020 0.025 0.024 0.1779 0.mer 0.515 0.514 0.1779 0.mer 0.515 0.7 0.440 0.172 0.444 0.7 0.440 0.372 0.444 0.372 0.444 0.7 0.440 0.372 0.444 0.372 0.444 0.769 0.266 0.2261 0.5726 0.5726 0.759 0.440 0.452 0.2546 0.357 0.96 0.466 0.546 0.357 0.546 0.975 0.466 0.547 0.546 0.357 0.966 0.546 0.547 0.546 0.547 0.975 0.946 0.546 0.547 0.546 0.972 0.944 0.946 0.543 0.544	000 0.028 0.025 40 0.244 0.1779 0.027 40.7 0.373 407 0.314 0.372 0.047 40.8 0.178 40 0.226 0.2211 0.129 0.2 8 0.079 40 0.226 0.2211 0.129 0.2 8 0.079 40 0.4612 0.260 0.007 0.0 0.079 40 0.4612 0.260 0.007 0.0 0.029 4.0 0.650 0.512 0.040 7.4 0.000 4.11 0.499 0.513 0.040 7.2	05 0.028 0.029 0.	05 0.020 104.004 0.0404 0.025 0.0244 0.1779 0.1827 60.7 0.0007 60.7 0.025 0.0244 0.1779 0.1827 60.7 0.0007 60.2 60.2 0.372 0.70 0.1827 8.844 9.8.5 44.55 60.2 0.169 40 0.2251 0.1205 42.2 60.2 60.2 0.169 40 0.2251 0.1205 42.5 60.2 60.2 0.170 40 0.401 0.2551 0.1205 43.100 60.5 0.0171 40 0.602 0.2551 0.1205 43.100 60.5 0.0170 40.0 0.602 0.512 0.006 97.931 60.5 0.025 4.40 0.602 0.512 0.006 97.931 60.5 0.020 4.41 0.789 0.513 0.007 72 80.5	05 0.020 0.025 0.	05 0.003 0.004 0.004 0.00404 </td <td>06 0.028 004004</td> <td>05 0.020 N04.001 N04.0</td> <td>05 0.025 0.</td> <td>05 0.028 0.029 0.</td>	06 0.028 004004	05 0.020 N04.001 N04.0	05 0.025 0.	05 0.028 0.029 0.

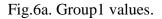


Fig.6b. Group2 values.

Croial Charles		minin	(((())))) (()) (()) (()) (()) (()) (()				- and - and - and - and	and great makes !	man many work					
Georgen3 (mgmd)										90014 90014 90014				
Sarrigein.	Conversitative	Walte	Mature 1	Malats Wallouts	Obd Dwe	CV#	Bankindon.	North Maria	11111111111	10211	num	ununn	111111112525	
16.9	2.000	1000	0.008	0.000	0.004	40.6	48.000	A state of the second	11/11/11/11/11	1119981	man	107HUU	A1111111111	
100	1.000	1000	0.011	0.000	0.000	200.1	102.445	all track and a straight	111-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-	[[]]符[111515	mm		
		(And []]]	0.079				00.329	CONTRACTOR -	11.1 State 1 1	11111	1111611	11111111	11111114403	
100.1	10.000	1895	1030162	0.078	8-067	4.8	47 090 81 1639	201000	T 1000 14 11	12221	111444	14999999	111111616	
-	0.00	616-0	17-03-70-82	1. 120	10.4147	12.00	25.460 J	and the second	The state of S	/////	111141	mm	111111111111111111111111111111111111111	
	and the second second	1121	1 (4) (4) (()	11111111	Land Land		79.800	00000000000	102531	125	111211	111111	11111110	
91.0		67,000,733 53,000,733	1020	0.242	0.12+#	8.81	10 228	and Company		11+1	mu	mm	numer	
		ener:	1 02886	11110.000	11.000	29.2	111	MARCH COM	2011111	,,,,		11111	uuun	
12/12/20	Transferral	0.6411	10-2006 10-707 10-707	1111 1000	0.071	1000	ALC: 1 1		ann			44440	mm	
1.1	960	12101		111111111	Concession in the	THE	501 148	······································			11114	ann a	<i>ttttt</i>	
int.	The second second	4/411	[[請罪]	0.944	-6.937	1243411	111111111111111111111111111111111111111							

Fig.6c. Group3 values.

Fig.6d. Group4 values.

Conclusion:

It can be concluded from the present study that all the three Gymnospermous essential oil have some activity against *S. typhimurium*. Taxodium oil shows remarkable efficiency over Pinus oil and Thuja oil against bacteria. Taxodium oil shows great efficiency against *S. typhimurium* and other microbes (21). The components (terpenes) of essential oil of *P. roxburghii* needles are highly active against microbes. As this oil significantly inhibited the growth of certain bacteria and fungi tested. The main oil component of *P. roxburghii* essential oil are monoterpene and sequiterpene hydrocarbons and their derivatives. These derivatives act as antibacterial and antifungal substance, the most well-known of which being terpenes and phenolics in general (22). The essential oil from the leaves and cones of bald cypress trees grown exhibited potent antimicrobial activities against bacteria (23). Essential oils from needles and foliages of these gymnosperms plants *viz.*, *P. roxburghii*, *T. distichum* and *T. occidentalis*, exhibit great potential eco-friendly, non-toxic, cost-efficient and antibacterial herbal formulations.

Acknowledgements:

I would like to give special thanks to Prof. Anupam Dikshit (FNASc), Director of Biological Product Laboratory (BPL), Department of Botany, University Of Allahabad, for providing me laboratory facilities; to Dr. S. k. Shukla; Mr. Saket Jha and Mr. Sharad Kumar Tripathi for their valuable suggestions.

References

 Press JR, Shrestha KK, Sutton DA. Annotated Checklist of the Flowering Plants of Nepal. The Natural History Museum. 2000

- 2. Wu Z, Raven PH. Flora of China. Vol. 4. Beijing Science Press; 1999.
- Gewali MB. Institute of Natural Medicine. Japan: University of Toyama; 2008. Aspects of Traditional Medicine in Nepal; pp. 19–20.
- Vallejo MCN, Evandro A, Sergio ALM. Volatile wood oils of the Brazilian Pinus caribaea var. hondurensis and Spanish Pinus pinaster var. mediterranea. J Braz Chem Soc. 1994;5:107– 112.
- 5. Asta J, Jurgita S, Aida S, Eugenija K. Characteristics of essential oil composition in the needles of young scots pine (*Pinus sylvestris* L.) stands growing along and ariel ammonia gradient. Chemija. 2006;17:67–73.
- Hassan A, Amjid I. Gas chromatography-mass spectrometric studies of essential oil of *Pinus roxburghaii* stems and their antibacterial and antifungal activities. J Med Plant Res. 2009;3:670–3.
- Adams RP (2001): Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Carol Stream, IL, Allurred Publishing Corporation, pp. 1–456.
- El Tantawy ME, El Sakhawy FS, El Sohly MA, Ross SA (1999): Chemical composition and biological activity of the essential oil of the fruit of *Taxodium distichum*. L. growing in Egypt. J Essent Oil Res 11: 386–392 (and references cited therein).
- 9. Flamini G, Luigi C, Morelli I (2000): Investigation of the essential oil of feminine cones, leaves and branches of *Taxodium distichum*. from Italy. J Essent Oil Res 12: 310–312.
- 10. Geiger H, de Groot-Pfleiderer W (1979): Die flavon- und flavonolglykoside von *Taxodium distichum*..Phytochemistry 18: 1709–1710
- Adams RP (2001): Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Carol Stream, IL, Allurred Publishing Corporation, pp. 1–456. [Google Scholar]
- 12. Denny, G.C. Evaluation of selected provenances of Taxodium distichum for drought, alkalinity and salinity tolerance. PhD Thesis, A&M University: Texas, 2007.
- 13. Kusumoto, N.; Ashitani, T.; Murayama, T.; Ogiyama, K.; Takahashi, K. Antifungal abietane-type diterpenes from the cones of Taxodium distichum Rich. J. Chem. Ecol., 2010, 36 (12), 1381-1386.
- Peng, D.; Wang, X.-Q. Reticulate evolution in Thuja inferred from multiple gene sequences: Implications for the study of biogeographical disjunction between easren Asia and North America. Mol. Phylogenet. Evol. 2008, 47, 1190–1202.

- Kamden, P.D.; Hanover, J.W. "Inter-Tree variation of essential oil composition of Thuja occidentalis L." J. Essent. Oil Res. 1993, 5, 279–282.
- 16. Duke, J.A. Handbook of Medicinal Herbs; CRC Press, Inc.: Boca Raton, FL, USA, 1985.
- 17. FAO (Food and Agriculture Organization of the United Nations). Non-Wood Forest Products from Conifers. Chapter 7-Essential Oils; FAO: Rome, Italy, 1995; Vol. 12, p. 86.
- Isiaka A. Ogunwande, Nureni O. Olawore, Oluranti O. Ogunmola, Tameka M. Walker, Jennifer M. Schmidt & William N. Setzer (2007) Cytotoxic Effects of Taxodium distichum. Oils, Pharmaceutical Biology, 45:2, 106-110
- 19. McFarland J. Nephelometer: an instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. J Am Med Assoc 1907; 14:1176-8.
- 20. Satyal P, Paudel P, Lamichhane B, Setzer WN. Volatile constituents and biological activities of the leaf essential of *Jasminum mesnyi* growing in Nepal. J Chem Pharm Res. 2012;4:437–9.
- Von Rudloff, E. Volatile Leaf Oil Analysis in Chemosystematic Studies of North American Conifers. Biochem. Sys. Ecol. 1975, 2, 131–167.
- 22. Gulten, T. G., Branden, A.N., Sahika, A. G., and Mehmat, K. (2012). "Antimicrobial activity of oregano oil on Iceburg lettuce with different attachment conditions." *J. Food Sci* 77(7), 412-415.
- 23. EI Tantawy, M.E.; El Sakhawy, F.S.; EI Sow, M.; Ross, S.A. Chemical Composition and Biological Activity of the Essential Oil of the Fruit of *Taxodium distichurn* L. Rich Growing in Egypt. *J. Essent. Oil Res.*, 1999, 11, 386-392.