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Synergistic Effect of Essential Oils of Three Gymnosperms Against *Vibrio Cholerae*

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Abstract: The present investigation focused on the determination of antibacterial efficacy of essential oils obtained from leaves of three gymnosperms i.e. *Pinus roxburghii* Sarg., *Taxodium distichum* L. and *Thuja occidentalis* L. The oils were extracted from the leaves of aforementioned plant species using Clevenger type apparatus by hydro-distillation method. The antibacterial activity of the extracted essential oils was evaluated against *Vibrio cholerae* (MTCC No. 3906) using broth micro-dilution method recommended by Clinical Laboratory Standard 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* Sarg., *T. distichum* L. and *T. occidentalis* L., were showed 1.294, 0.914 and 1.277 mg/ml respectively. The *P. roxburghii*, was found most effective with their MIC 1.403 mg/ml while *T. distichum* L., showed least effective with their MIC 1.995 mg/ml against *V. cholerae*. Hence, essential oil from leaves of gymnosperms exhibit great potential for the development of eco-friendly, non-toxic, cost-effective anti-bacterial formulations.

Key words: Gymnosperms, Essential oil, Synergistic effect, Broth Micro-dilution assay

I. INTRODUCTION

P. roxburghii Sarg. belongs to Pinaceae and commonly known as Chir pine. *P. roxburghii* is the native of Himalayas and distributed throughout India, Nepal, Bhutan and Pakistan. It is widely distributed from lower to midrange of Himalaya in India. *P. roxburghii* is a large tree attaining up to 55 m in height with a trunk diameter reaching up to 2 m (figure 1). 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 blisters, cuts, boils and wounds (2). Phytochemical screening of *Pinus* needles revealed the abundant amounts of tannins, vitamin C 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) as well as antibacterial activity (7) while alcoholic extract of the needle, stem, and cones are reported to exhibit strong anti-bacterial activity. *P. roxburghii* grows in the region of forests of 1200-1850 m altitude at a temperature between 5-15°C (8).

Taxodium distichum (L.) belongs to Taxodiaceae and commonly referred as bald cypress. It is an unusual but interesting tree. It can grow over 25 m in height and over 3 m in diameter (figure 2). The leaves are small, green to yellow-green, 5–20 mm long and appeared in two-ranks. Young trees have a pyramid shape but eventually form an irregular flattened canopy. Cones are the fruits and are composed of scales forming a woody, brown sphere with rough surface. *T. distichum* has three extant taxa ranging from the eastern United States through Mexico to Guatemala (9). Heartwood of bald cypress is extremely rot and termite resistant (10). Leaves and cones are rich in essential oils and used traditionally to treat respiratory, skin, gastro-intestinal, inflammation, and infections (11, 12). Diterpenoids and Flavonoids are the main secondary metabolites (13). *T. distichum* trees can grow near lake margins, rivers, swamps, wet poorly drained habitats and are tolerant to various soil conditions and air pollution (14). These long-lived conifers have been widely used for landscape in many countries. The heartwood is used for building materials, and has been reported to resist the attacks of the subterranean termite (15).

Fig1. *P. roxburghii*

Fig 2. *T. distichum*

Fig 3. *T. occidentalis*

Thuja occidentalis L. belongs to Cupressaceae and commonly known as White Cedar and Morpankhi in Hindi. It is native to Eastern Canada and other regions on United State; widely cultivated as an ornamental plant throughout the planet (figure 3). *T. occidentalis* has been used to treat uterine carcinomas, bronchial catarrh, psoriasis and rheumatism (16). The essential oil of the plant has been used for soft soaps, room sprays, disinfectants and insecticides. Cedar leaf oil can be obtained by hydro distillation of the foliage and is used for the production of perfumes, insecticides, soaps and deodorants (17, 18). The essential oil is an active ingredient in the production of antibacterial herbal formulations, cough suppressants, soap and perfumes, while many cultivars are grown for ornamental purposes (7, 19). The oil of eastern cedar leaves (*T. occidentalis*) has been independently investigated by Shaw (20) and Rudloff (20), who reported the thujone fraction as a mixture of Z-thujone and E-isothujone, while Keita et al. (21) in their analyses, reported twenty-two compounds including α -thujone (= Z-thujone) (49.64%), fenchone (14.06%) and β -thujone (= E-isothujone) (8.98%) as the most abundant compounds.

II. MATERIAL AND METHOD:

A. 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. Plant were identified at Department of Botany, University of Allahabad(7). Leaves were crushed and loaded into a Clevenger type Apparatus for hydrodistillation for 4-5 hours at 45 degree Celsius (7). 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. Oil content was stored at 4°C until analysis (7, 22).

Fig4 and 5. Clevenger type apparatus.

Fig6. Extracted oils

B. Preparation Of 0.5 Mcfarland Solution And Saline Media

On adding 1% of BaCl_2 to the freshly prepared solution of 200 ml of 1% H_2SO_4 , 0.5 McFarland solution is formed (7, 23). Now prepare saline media by dissolving 1 gm of NaCl into 100 ml of DDW (7). Take the O.D. of this saline media and McFarland solution.

C. Preparation of Mueller-Hinton broth (MHB) and Inocula

Take 1000 ml of DDW in a beaker. Add 21 gms of MHB powder. Shake well and boil up to 100°C . Close the mouth with sterilized synthetic plug (7). Inocula were prepared by using saline media and bacteria (7).

D. 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 were used for broth microdilutions. Column-1 contains 190 μL and 10 μL of formaldehyde and known as negative control (7). Column-2 contains 200 μL of MHB and known as broth control (7). Column-3 contains 180 μL broth and 20 μL drug in each row and known as drug control. Row A and B of column-3 contains 20 μL of Streptomycin. Row C and D contains 20 μL of chir oil. Row E and F contains bald cypress oil whereas row G and H contains cedar oil. Now add 100 μL of broth from column-4 to column-12. In column-4, add 80 μL broth and 20 μL drugs in each row as mentioned previously. Now homogenize and dilute the drugs 1:1 in MHB horizontally from column-4 to column-11. Finally add 100 μL inocula from column-4 to column-12 (7) (figure 7). Final volume of each well were 200 μL . The solutions were incubated at 37°C for 24 hours (7, 24). *Streptomycin* used as positive control. Formaldehyde was used as a negative control.

Fig7. – Diagrammatic representation of horizontal broth microdilution.

III. RESULTS

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 1.294, 0.914 and 1.277 mg/ml respectively (Figure 8, 9). The minimum inhibition concentrations (MIC) of *P. roxburghii*, *T. distichum* and *Thuja occidentalis* were recorded 1.403, 1.995 and 1.864 mg/ml respectively (Figure 8,9). *P. roxburghii* was found to be most effective with their MIC 1.403 mg/ml whereas *T. distichum* was found to be least effective with their MIC 1.995 mg/ml against *V. cholerae* (Figure 9).

Fig8. Graph obtained for antibacterial activity of essential oils and values of IC50 and MIC.

Fig9. Different values of different drugs against V. cholerae obtained from SpectramaxPlus 384 molecular Device USA.

V. CONCLUSION

It can be concluded from the present study that all the three Gymnospermous essential oil have some synergistic activity against V. cholerae. It was first time in our best knowledge that essential oil of P. roxburghii shows remarkable effect against V. cholerae. Chir oil shows remarkable efficiency over bald cypress oil and white cedar oil against bacteria. Pinus oil shows great efficiency against V. cholerae and other microbes (25). 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 tested. The main oil component of P. roxburghii essential oil are monoterpene and sesquiterpene hydrocarbons and their derivatives. These derivatives act as antibacterial and antifungal

substance, the most well-known of which being terpenes and phenolics in general (26). The essential oil from the leaves and cones of bald cypress trees grown exhibited potent antimicrobial activities against bacteria (27). 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.

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REFERENCES

- [1] 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.
- [3] Gewali MB. Institute of Natural Medicine. Japan: University of Toyama; 2008. Aspects of Traditional Medicine in Nepal; pp. 19–20.
- [4] 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.
- [6] Hassan A, Amjid I. Gas chromatography-mass spectrometric studies of essential oil of *Pinus roxburghii* stems and their antibacterial and antifungal activities. J Med Plant Res. 2009;3:670–3.
- [7] Ravikant Singh, Ashutosh Pathak, Anupam Dikshit, Rohit Kumar Mishra, "COMPARISON OF BIOLOGICAL ACTIVITIES OF ESSENTIAL OIL OF THREE GYMNOSPERMS AGAINST SALMONELLA TYPHIMURIUM", INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT), ISSN:2320-2882, Volume.6, Issue 1, Page No pp.1420-1426, February 2018, Available at : <http://www.ijcrt.org/IJCRT1802181.pdf>
- [8] Sidiqi KM, Iqbal M and Mohammad A. Forest ecosystem climate change impact assessment and adaptation strategies for Pakistan. Clim. Res., 1999; 12: 195-203.
- [9] Adams RP (2001): Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Carol Stream, IL, Allured Publishing Corporation, pp. 1–456.
- [10] 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).
- [11] 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.
- [12] Geiger H, de Groot-Pfleiderer W (1979): Die flavon- und flavonolglykoside von *Taxodium distichum*. Phytochemistry 18: 1709–1710
- [13] Adams RP (2001): Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Carol Stream, IL, Allured Publishing Corporation, pp. 1–456.
- [14] Denny, G.C. Evaluation of selected provenances of *Taxodium distichum* for drought, alkalinity and salinity tolerance. PhD Thesis, A&M University: Texas, 2007.
- [15] 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.
- [16] Peng, D.; Wang, X.-Q. Reticulate evolution in *Thuja* inferred from multiple gene sequences: Implications for the study of biogeographical disjunction between eastern Asia and North America. Mol. Phylogenet. Evol. 2008, 47, 1190–1202.
- [17] 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.
- [18] Duke, J.A. Handbook of Medicinal Herbs; CRC Press, Inc.: Boca Raton, FL, USA, 1985.
- [19] 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.
- [20] Von Rudloff, E. Gas-liquid chromatography of terpenes VI. The volatile oil of *Thuja plicata* Donn. Phytochemistry 1962, 1, 195–202.
- [21] Keita, M.S.; Vincent, Ch.; Schmidt, J.-P.; Arnasson, J.T. Insecticidal effects of *Thuja occidentalis* (Cupressaceae) essential oil on *Callosobruchus maculatus* (Coleoptera: Bruchidae). Can. J. Plant Sci. 2001, 81, 173–177.
- [22] 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
- [23] 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.
- [24] 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.
- [25] Von Rudloff, E. Volatile Leaf Oil Analysis in Chemosystematic Studies of North American Conifers. Biochem. Sys. Ecol. 1975, 2, 131–167.
- [26] Gulen, 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.
- [27] El Tantawy, M.E.; El Sakhawy, F.S.; El Sow, M.; Ross, S.A. Chemical Composition and Biological Activity of the Essential Oil of the Fruit of *Taxodium distichum* L. Rich Growing in Egypt. J. Essent. Oil Res., 1999, 11, 386-392.

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