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

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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*. IC_{50} and Minimum Inhibition concentrations (MIC) using SpectramaxPlus^{384}, of Molecular Devices Corporation, USA were recorded while Streptomycin as standard was taken. The IC_{50} 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 long-lived 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% $BaCl_2$ 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 ºC. Close the mouth with cotton plug. Place the solution inside autoclave. After this, MHB is ready to use.

Preparation of inocula – 750 µL of saline media was taken in a culture tube. Add 250 µL of bacteria in the same tube. Final volume in the culture tube is 1000 µL. Now take 500 µL 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 µL and 10 µL of formaldehyde (added after the completion of microdilution). Column-2 contains 200 µL of MHB. Column-3 contains 180 µL broth and 20 µL drug in each row. Row A and B of column-3 contains 20 µL of streptomycin. Row C and D contains 20 µL 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 µL of broth were added from column-4 to column-12. In column-4, add 80 µL and 20 µL drugs in each row one by one as described previously. Now dilute the drugs horizontally from column-4 to column-11. Now add 100 µL inocula to each well from column-4 to column-11. Final volume of each well were 200 µL. 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.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).

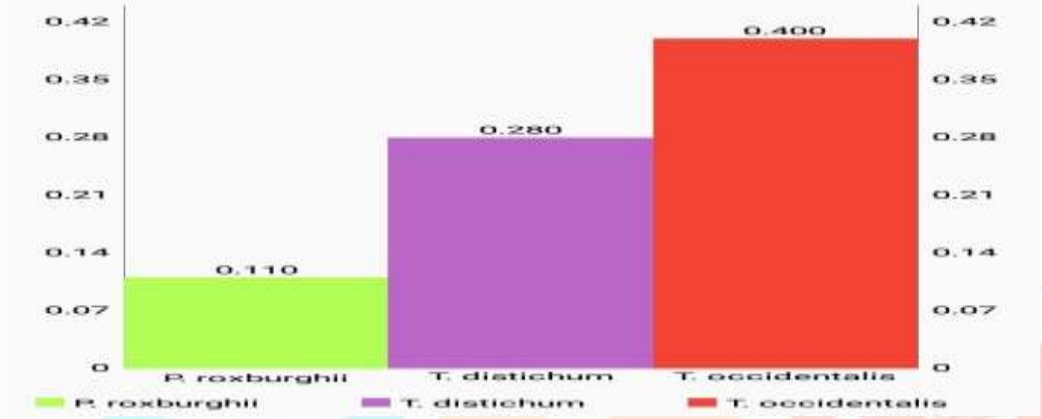

Fig.4. Percentage yield of essential oils.

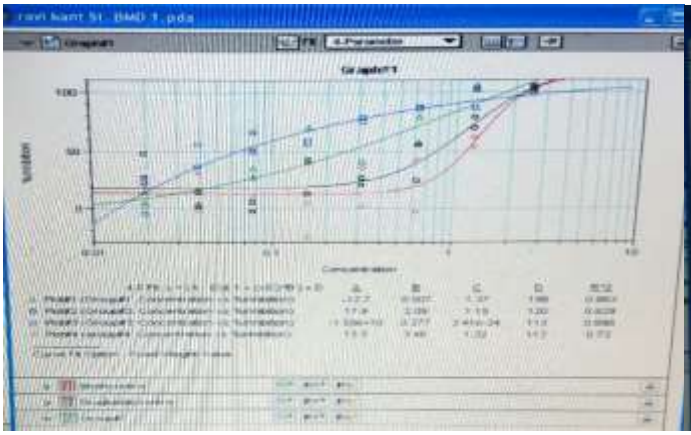

Fig.5a. Graph showing of IC50 and MIC.

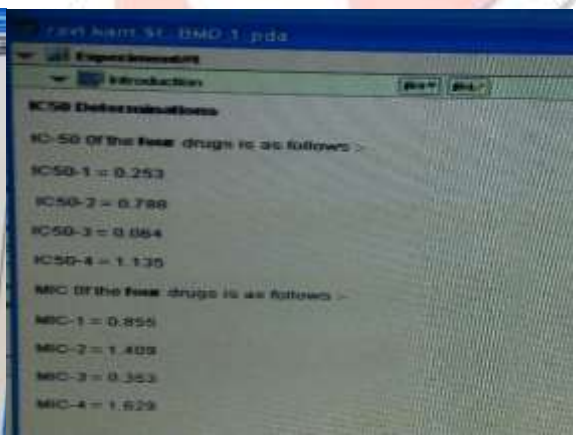

Fig. 5(b). IC50 and MIC values.

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).

Fig.6a. Group1 values.

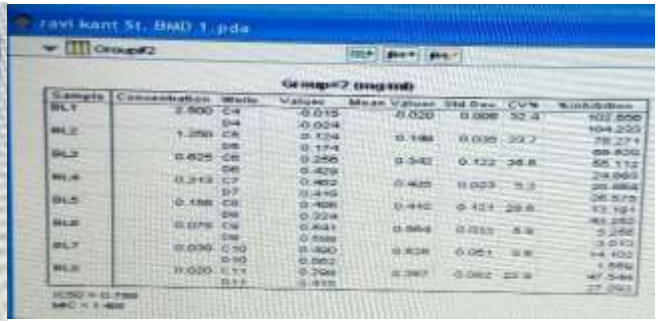

Fig.6b. Group2 values.

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.

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