# Comparison of Antibacterial Activities of essential oils of *Juniperus communis* L., *Pinus roxburghii* Sarg. and *Taxodium distichum* L. against *Klebsiella pneumoniea*

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**Abstract**

The present investigations were focused on the comparision of biological activities of the essential oils of the three gymnospermous foliages *i.e., Juniperus communis L., Pinus roxburghii* Sarg. and *Taxodium distichum* L., against *Klebsiella pneumoniea* (MTCC-4032). The oils were extracted from the leaves of aforementioned plant species using hydro-distillation method. The antibacterial activity of the extracted essential oils was evaluated against *Klebsiella pneumoniea* (MTCC-4032) 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 SpectramaxPlus384 Molecular Devices were recorded. Streptomycin as standard was taken. The $IC_{50}$ value of *J. communis, P. roxburghii,* and *T. distichum* were shown to be 0.051, 0.782 and 0.295 mg/ml respectively. The *J. communis* was found to be most effective with their MIC 0.117 mg/ml while *P. roxburghii* found to be least effective with their MIC 1.691 mg/ml against *K. pneumoniea*. Hence, essential oils of aforementioned gymnosperms exhibit great potential for the development of eco-friendly, non-toxic, cost effective, anti-bacterial formulations.

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

## Introduction:

*Juniperus communis* L. belongs to family Cupressaceae and commonly known as juniper (figure 1c). *J. communis* distributed throughout the cold temperate Northern Hemisphere including America, Europe and Asia. The essential oil, infusions, decoctions, and alcoholic extracts were used in different fields (pharmaceuticals, alcoholics, etc.) (6, 7, 9, 25-28). Changes in the composition of an essential oil can be caused by environmental factors, such as soil or climate in which the plants were grown and by different harvesting methods or distillation techniques. In the past few years, number of publications have reported the composition of the berries and leaves essential oil of the *Juniperus* species (28-32).

*P. roxburghii* Sarg. belongs to the family Pinaceae and commonly known as chir pine. *P. roxburghii* native of Himalaya and distributed throughout India, Nepal, Bhutan and Pakistan. It is widely distributed in western Himalayan region of India. *P.roxburghii* is a large tree attaining up to 25-55 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). Phytochemical screening of *Pinus* needles and stems unveil 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 of *P. roxburghii* has shown significant anti-fungal and anti-bacterial activity (6, 7, 10) 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) *J. communis*

*Taxodium distichum* L. belongs to Cupressaceae and commonly known as bald cypress. *T. distichum* is an unusual and interesting tree, often growing over 28 m in height and over 350 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 buteventually 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 (figure 2a, b, c). *T. distichum* has three extant taxa found in the eastern United States, Mexico and Guatemala (11). The trees are used for their wood because heartwood is extremely rot and termite resistant (12). Essential oils of leaves and cones were found to be effective against bacteria and usedtraditionally to treat gastro-intestinal, skin, respiratory, inflammation, and infections (9, 13, 14). Flavonoids and diterpenoids are the main secondary metabolites (14). *T. distichum* trees can grow on rivers, lake margins, swamps, wet poorly drained habitats and are tolerant to various soil conditions and air pollution (15). These long-livedconifers have been widely used for landscape in many countries. The heartwood of bald cypress is used forbuilding materials, and has been reported to resist the attacks of the subterranean termite (16).

Figure2. (a) Female cone (b) leaves (c) male cone of *T. distichum*.

**Material and method**:

**Extraction of essential oil** - The plant materials of *J. communis*, *P. roxburghii*, and *T. distichum*were collected from Roxburgh Garden, Department of Botany, University of Allahabad, in the month of December. Plants were identified at Department of Botany, University of Allahabad.


Figure3. Extracted oils (BPL1=juniper oil, BPL2=chir oil, BPL3=bald cypress oil).

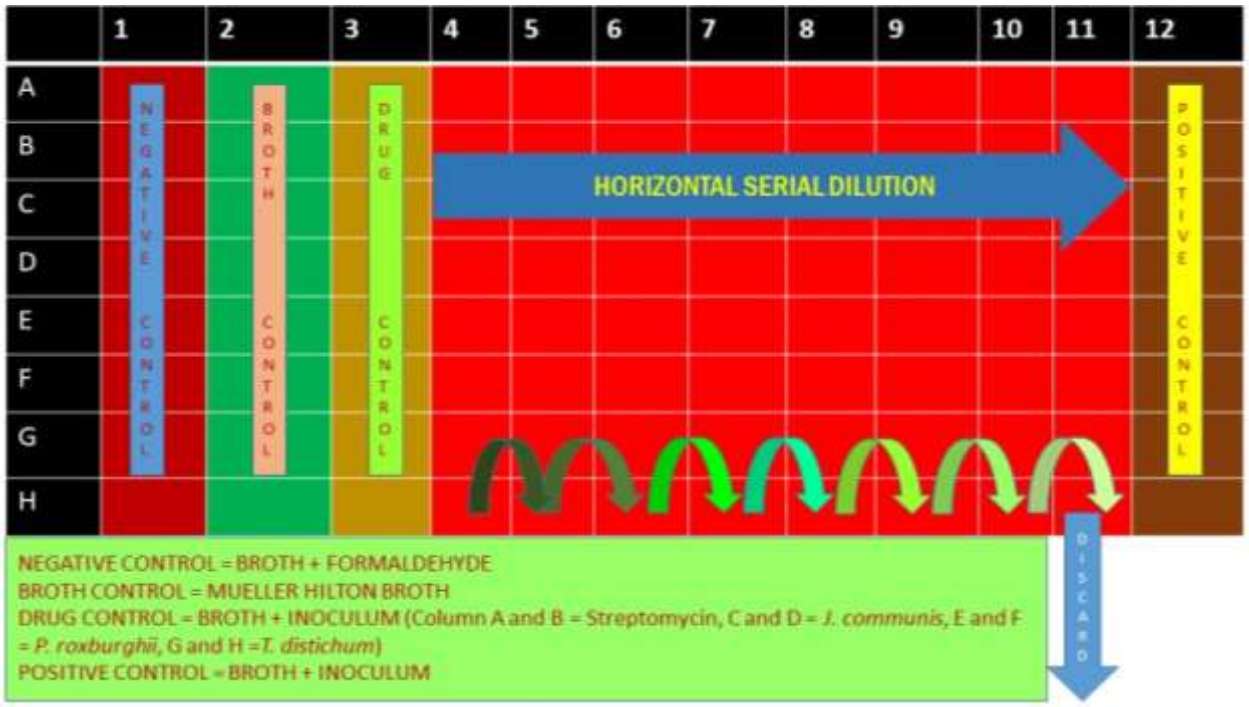
Leaves (needles, foliages) and branchletswere crushed and hydrolyzed using a Clevenger type Apparatus for 4-5 hours at 40°C. Essential oils of *J. communis* (common juniper) is transparent, *T.distichum* (bald cypress) appears as dark yellow in colour whereas oil of *P. roxburghii* (chir pine) i.e., pale yellow (figure 3). Oil content was stored at 4°C until analysis (7-9,18).

**Preparation of Mueller-Hinton broth (MHB) –** Take 500 ml of DDW in a beaker. Add 10.5gms 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-** Inocula of procured culture of pathogenic bacteria (48 hours old) was prepared comparing 0.5 McFarland Standard Solution by using Spectrophotometer at 480nm.

**Antibacterial Screening**- Essential oils were screened for antibacterial activity against *K. pneumonea* Minimum Inhibitory Concentrations (MIC) were determined using Broth Micro-dilution method recommendedby Clinical Laboratory Standard Institute (CLSI).

Figure 4:



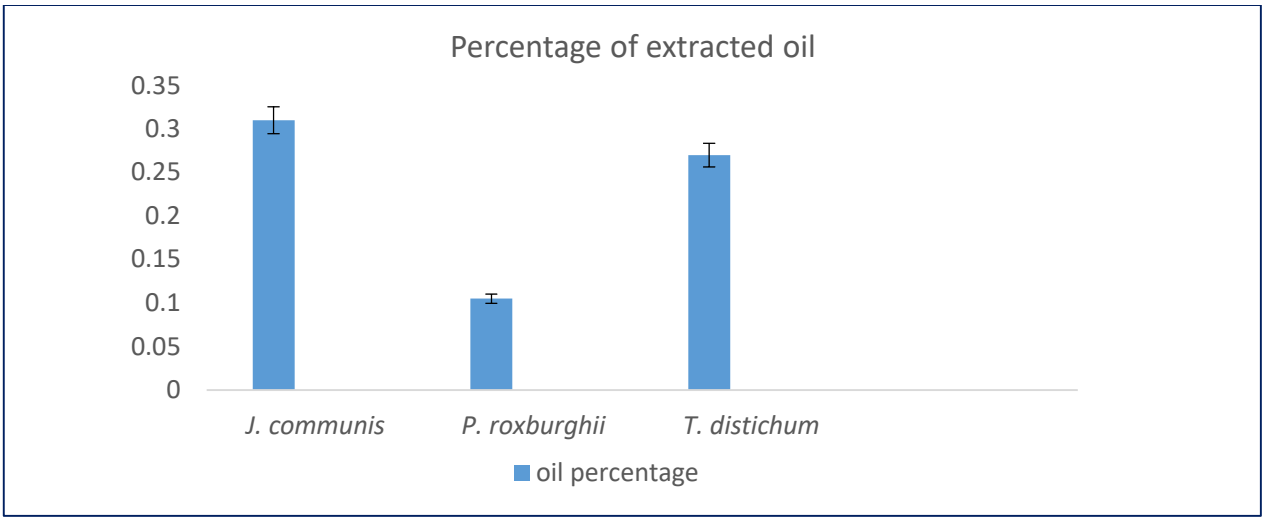

Diagrammatic representation of Broth micro dilution.

96 well plate was used for micro dilution. The Broth Micro-dilution protocol was used as recommended by CLSI- Antibacterial assay. Column-1 contains formaldehyde and is known as negative control. Column-2 contains MHB as broth control. Column-3 and Column-4 contains drug in each row. Row A and B of streptomycin. Row C and D contain *Juniperus* oil. Row E and F contain *Pinus* oil whereas row G and H contains *Taxodium* oil. Column-3 is known as column of drug control. Now dilute the drugs horizontally from column-4 to column-11 by using multichannel micropipette (figure 4). Column-12 was filled with bacterial inoculum as positive control. The extract solutions over horizontally diluted 1:1 in MHB in a 96 well plates were incubated at 37 °C for 24 hours (7-9, 20). Inhibitory concentration and it was determined as the lowest concentration without turbidity. Streptomycin used as Drug (Standard) control. Formaldehyde was used as a negative control.

**Results:**

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

*J. communis = 0.31%, P. roxburghii* = 0.105 % and *T. distichum* = 0.270% (graph 1).



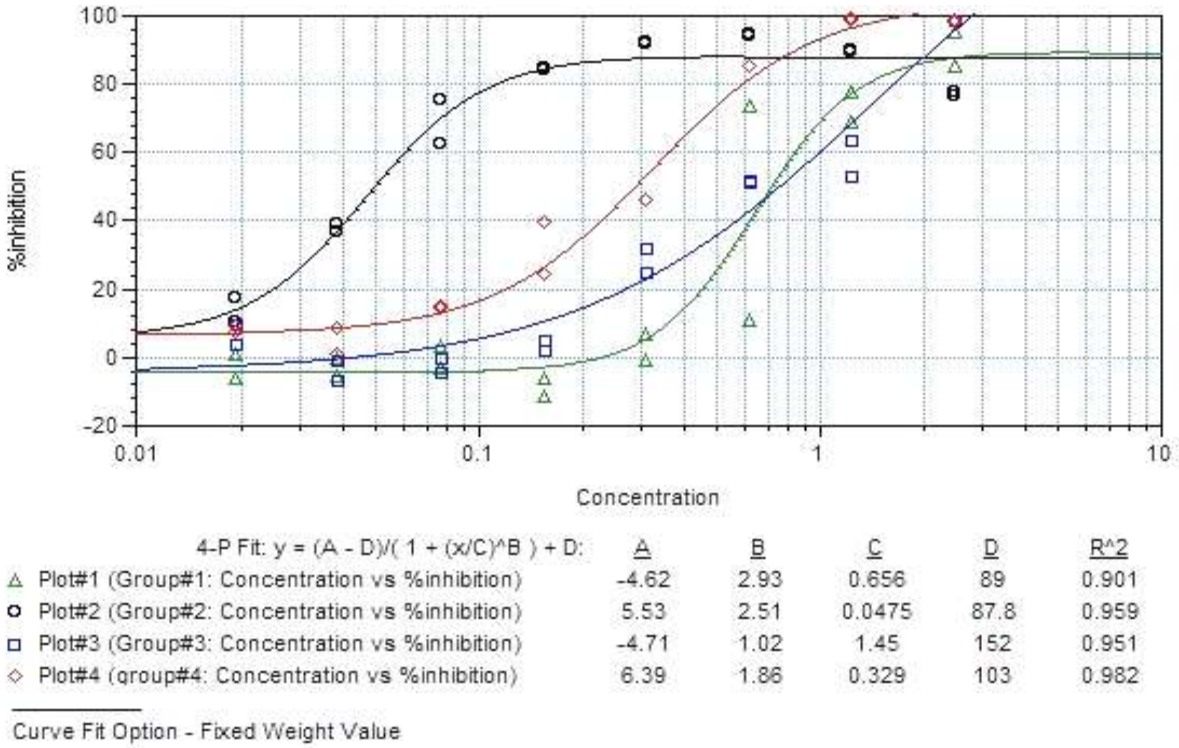

Graph 1: Percent yield of essential oils of aforementioned Gymnosperms.

Table1. IC-50 0f the four drugs is as follows:-

| S.NO. | IC50 | Values |
|---|---|---|
| 1 | IC50-1 | 0.736 |
| 2 | IC50-2 | 0.051 |
| 3 | IC50-3 | 0.782 |
| 4 | IC50-4 | 0.295 |

Table2. MIC 0f the four drugs is as follows:-

| S.NO. | MIC | Values |
|---|---|---|
| 1 | MIC-1 | 1.409 |
| 2 | MIC-2 | 0.117 |
| 3 | MIC-3 | 1.691 |
| 4 | MIC-4 | 0.609 |




Graph 2: Concentration vs %inhibition.

The results were recorded in terms oil Inhibition Concentrations ($IC_{50}$) and Minimum Inhibition Concentrations (MICs) via Spectramax Plus384, Molecular Devices Corporation, USA (graph 2). $IC_{50}$ value of *J. communis*, *P. roxburghii* and *T. distichum* were showed 0.051, 0.782 and 0.295 mg/ml respectively (table 1). The minimum inhibition concentrations (MIC) of *J. communis*, *P. roxburghii* and *T. distichum* were recorded 0.117, 1.691 and 0.609 mg/ml respectively (table 2). *J. communis* was found to be most effective with their MIC 0.117 mg/m1 whereas *P. roxburghii* was found to be least effective with their MIC 1.691 mg/ml against *K. pneumoniea*.

**Conclusion:**

It has been concluded from the present study that all the three Gymnospermous essential oil have antibacterial activity against *K. pneumoniea*. Juniper oil shows remarkable efficiency over chir oil and cypress oil against bacteria. *Taxodium* oil shows great efficiency against *S. typhimurium* and other microbes (6, 9, 21). Essential oils from needles and foliages of these gymnosperms plants *viz.*, *J. communis*, *P. roxburghii* and *T. distichum* exhibit great potential eco-friendly, non-toxic, cost-efficient and antibacterial herbal formulations.

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