

1 **Assessment of total phenolic and flavonoid contents and potential biological efficacy of**
2 **few *Pinus* species growing in Northern Himalayas.**

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43 **Abstract:**Environmental interventions and ecological adaptations harbor millions of valued
44 substances and metabolites in plants which can be employed and commercialized for human
45 benefits. Present study encompasses the untapped potential of pine needles of Indo-
46 Himalayan region for the production of different metabolites and their pharmacological
47 significance in terms of antioxidant and antimicrobial activity. Total phenolic and flavonoid
48 content from the needles of ten pine species was quantified using three different solvent
49 systems. Results revealed that out of 10 different selected *Pinus* species, *Pinus taeda* showed
50 highest concentration of total phenolics, soluble-F phenolics and flavonoids content
51 (approx.147.02 mg/g, 141.08 mg/g and 21.91mg/g respectively) as compared to other species.
52 On the other hand *P. greggii* showed highest Bound-W phenolic content (approx.3.62mg/g).
53 Among all the selected plant species, the needles of *P.echinata* exhibited the highest and
54 *P.thunbergii* had the lowest ratio of total flavonoids to total phenolics. Most of these
55 compounds were found to have effective antioxidant activities as well as antimicrobial
56 activity, as estimated by oxygen radical absorbance capacity (ORAC) and disk diffusion test
57 respectively.

58 Keywords: *Pinus*, needle, Himalayas, Phenolics, Flavonoids, Antimicrobial, Antioxidant.

59 1. Introduction

60 In order to protect themselves against an array of pathogenic attacks and oxidative stress,
61 plants produce diverse phytochemicals called as secondary metabolites. Such secondary
62 metabolites possess several ecological and biological activities viz., anti-inflammatory, anti-
63 oxidant or antimicrobial. In addition they also exhibit a wide spectrum of actions such as anti-
64 mutagenic, antitumor as well as an protectant against oxidative DNA damage, and apoptosis
65 induced by hydroxyl radicals^{1,2,3}. These compounds also play an important role in imparting
66 attractive colours to flowers that help in attracting pollinating agents. Besides their biological,
67 nutraceutical and clinical significance, flavonoids are also involved in various plant defense
68 mechanisms⁴. Such bioactive defense compounds are also important when it comes to plant
69 responses against environmental hazards, such as pollution, temperature fluctuations and UV
70 radiations⁵. Phenolic compounds show great variations after high temperature shocks to stems
71 and crown, proving their importance as bioindicators of thermal stress⁶. These plant based
72 metabolites are of enormous scientific interest. Phenolics and flavonoids are the largest
73 category of such phytoconstituents and are most common. The use of phenolic and flavonoid

74 based plant metabolites requires large-scale sources of these plants to meet ever increasing
75 industrial demands.

76 The Himalayas, recognized for its ecosystem services is one of the hotspots of biodiversity
77 and is also one of the richest ecosystems on earth having a variety of species and forest types
78 due to the varying climatic conditions. They harbor more than 8,000 species of vascular
79 plants, of which 25.3% are endemic⁷ and cover about 12.84% of the total geographical area
80 of India⁸. With over 105 species, *Pinus* is the most dominant genus of the family Pinaceae
81 belonging to gymnosperms and has a wide range of distribution and adaptability to diverse
82 climatic conditions in Northern as well as Southern Hemisphere on the globe⁹. The pine
83 species growing in Himalayas are no doubt a unique plant suiting these pharmaceutical and
84 industrial needs. From earliest times, this genus has been of great economic value and
85 ecological significance. The value of this genus further increased with the development of
86 industrial technologies as pines turned out to be an excellent source of oleoresin, which after
87 processing yielded turpentine and colophony and pine needles and pine sprouts being raw
88 materials for pine teas¹⁰. Most of the previous works done are either associated with
89 chemistry and pharmacognotic aspects of bark or chemical composition and antimicrobial
90 activity of essential oil distilled from needles of various pine species and their therapeutic
91 value. Despite the increase in scientific data on the phenolic and flavonoid profile of pine
92 species, a literature search revealed lack of information on *Pinus* species from the Indian
93 Himalayan flora. Thus, the main objective of the current study was to quantify the total
94 phenolics and flavonoids of ten selected species of *Pinus*. This study also examined the
95 biological efficacy in different extracts of pine needles by assessing their antioxidant and
96 antimicrobial capacity. The aim of study was to gather more information that may further
97 clarify the role of secondary metabolites of *Pinus* in medicine and other commercial
98 applications.

99 2. Materials and methods

100 2.1. Collection and authentication of plant material

101 The leaves (needles) of 10 pine species (Out of these ten, five species *P.merkusii*, *P.khasya*,
102 *P.thunbergii*, *P.wallichiana* and *P.roxburghii* are native to India and other five (*P.taeda*,
103 *P.elliottii*, *P.echinata*, *P.patula* and *P.greggii* are introduced or exotic and now growing
104 luxuriantly) were collected in September, 2016, from a wild population in the region of
105 Ranikhet (located at 357 km NSE of New Delhi: latitude 29°39'52.2" (N); longitude
106 79°28'40.9" (E); altitude 1,727 m). The site is characterized by an average temperature of

107 14.4 °C, median rainfall (about 1575 mm of precipitation annually) and low soil fertility. A
108 voucher specimen of all the species selected for study was deposited in the herbarium of the
109 National Botanical Research Institute, Lucknow and identified.

110 **2.2 Extract preparations and Estimation of total Phenolic and flavonoid contents**

111 Pine needle extracts and fractions (Soluble free, Bound ethyl acetate and bound water) were
112 prepared by the method of Sun et al., (2002)¹⁰. All the three fractions were stored at -80°C
113 prior to estimation of total phenolic and total flavonoid content. Experiment was executed in
114 triplicate.

115 **2.3 Chemicals**

116 Folin-Ciocalteu reagent, gallic acid, aluminum chloride and naringin were purchased from
117 Sigma-Aldrich (United States) and ethyl alcohol, ethyl acetate, hydrochloric acid, sodium
118 carbonate, sodium hydroxide, phosphate buffer (pH 7.0) and trolox reagent from Gyan
119 Scientific (Lucknow, India).

120 **2.4 Estimation of total Phenolic**

121 Total phenolic content was estimated in a 96-well plate as per method of Zhang et al.,
122 (2006)¹². 100µl of the Folin-Ciocalteu reagent was added to 5 µl of needle extract
123 (concentration 1µg/µl), mixed thoroughly and kept for 10 minutes, followed by addition of
124 80µl of 7.5% sodium carbonate. The plate was then left for 2 hours in the dark at room
125 temperature. Absorbance was measured at 750nm on UV-vis spectrophotometer (Spectra
126 scan UV 2700, Thermo scientific). Gallic acid was used as standard in this estimation of
127 phenolic content. Results were expressed as mg of gallic acid equivalent (GAE) per gram of
128 extract. Blanks were prepared for both the standard as well as extracts by replacing gallic acid
129 and plant extract with milliQ water respectively.

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131 **2.5 Calculations**

132 A standard curve was constructed with gallic acid of concentration ranging from 0.5µg/ml to
133 4µg/ml. The concentration of all the unknown samples were then calculated based on the
134 equation of the standard curve which was

$$135 \quad y = 0.276x + 0.104 \text{ (Figure 1).}$$

136

137 **2.6 Estimation of total flavonoid**

138 Total flavonoid content in the needle of all the selected *Pinus* species were estimated as per
139 the method of Chang et al., (2002)¹³. 200µl of needle extract with concentration (1µg/µl) was
140 added to 1.5ml of 95% ethyl alcohol. To this 0.1ml of 10% aluminium chloride was added

141 followed by addition of 0.1ml of 1M potassium acetate and 2.8 ml of distilled water. The
142 mixture was then incubated for 25 minutes at +25°C and the absorbance was recorded at
143 415nm using UV–vis spectrophotometer (Spectra scan UV 2700, Thermo scientific). The
144 result was expressed as mg of Naringin equivalent per gram of needle extract.

145 **2.7 Calculation**

146 Naringin was used as a standard in this estimation. A standard curve was constructed with
147 Naringin of concentration ranging from 5µg to 25µg in the reaction mixture. The
148 concentration of all the unknown samples were then calculated based on the equation of the
149 standard curve which was

$$150 \quad y=0.01x+0.013 \text{ (Figure 1).}$$

151 **2.8 Antioxidant activity**

152 The oxygen radical absorbance capacity (ORAC) of each fraction in selected pine needle
153 extracts was measured using the method given by Yoon et al., (2010)¹⁴. All the three fractions
154 were diluted with 75 mM phosphate buffer (pH 7.0) and a standard curve was obtained by
155 plotting four concentrations of Trolox against the net area under the curve of each standard.
156 Final calculation of ORAC values was done using the regression equation between Trolox
157 concentration and AUC (Net area under curve). Results are expressed as µmole of Trolox
158 equivalents per gram dry weight.

159 **2.9 Antimicrobial activity**

160 **2.9.1 Microbial strains**

161 All the three fractions (Soluble free, bound ethyl acetate and bound water) were separately
162 tested against a panel of eight pathogenic microorganisms including six bacterial strains (out
163 of which two are gram positive namely *Staphylococcus aureus* (MTCC 96), *Streptococcus*
164 *mutans* (MTCC 890) and remaining four are gram negative namely. *Klebsiella pneumoniae*
165 (MTCC 109) *Escherichia coli* (MTCC 739), *Salmonella typhimurium* (MTCC 98),
166 *Pseudomonas aeruginosa* (MTCC 741) and two fungal strains (*Candida albicans* (CA-3010)
167 and SS=*Sporothrix schenckii*). All microorganisms were obtained from the Microbiological
168 laboratory, Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow.

169 **2.9.2 Disc diffusion assay**

170 Antimicrobial activities of three extracts were initially screened for their inhibitory zone
171 using agar disc diffusion assay. The microbial suspension of bacterial and fungal strains
172 under study was prepared by diluting the cell mass in saline water and adjusting it to 0.5
173 McFarland scale. 20 mL of sterile agar [Sabouraud dextrose agar for fungal strains and
174 Mueller Hinton for bacterial strains] were poured in the petri plates and it was allowed to dry.

175 The inoculums were inoculated using Streak plating technique over the media. A sterile paper
176 discs (5.5 mm of paper) soaked with 10 µlitre of the different plant extracts (100 mg/mL)
177 were added in the petri dish. Before incubation, all petri dishes were stored in the dark at
178 +4°C for 2 hours to facilitate the diffusion of the extracts into medium without microbial
179 growth. After incubation at 37°C/24 hours for bacterial strains and 30°C/48 hours for fungal
180 strains, activity was recorded by measuring the diameter of inhibitory zone (in mm). The
181 result represents average of three replicates.

182 2.9.3 Minimum inhibitory concentration (MIC)

183 MIC is defined as the minimum concentration of the extract at which growth inhibitory
184 activity can be seen. In present study it was determined by the agar dilution method as
185 described by Ebrahimabadi et al., (2010)¹⁵. Plant extract was added to the sterile medium and
186 the resulting agar solutions were mixed and poured into the petri dishes. The plates were
187 inoculated with 1 µl of selected bacterial and fungal strains.

188 3. Results and Discussion

189 Preliminary phytochemical analysis of various solvent extracts such as extract soluble free
190 (Soluble-F), bound ethyl acetate (Bound-E) and bound water (Bound-W) extract in needles of
191 ten species of *Pinus* revealed the presence of several phenolics and flavonoids in varying
192 quantities. These extracts show growth inhibitory activity against tested strains of bacteria
193 and fungi.

194 3.1 Total phenolic content

195 The total phenolic content (Table 1 and Figure 2) in needles of ten pine species was estimated
196 using Gallic acid as standard. Phenolic content was expressed as Gallic Acid equivalent
197 (GAE)/g of tissue. Among all pine needles analyzed, *P.taeda* had the highest Soluble-F
198 phenolic content (141.08±2.34mg/g), followed by *P.thunbergii* (139.67±0.89mg/g). *P.khasya*
199 had the highest Bound-E phenolic content (4.87±0.22mg/g) while highest Bound-W phenolic
200 content (3.62±0.23 mg/g) was reported in *P.greggii*. *P.taeda* had the highest total phenolic
201 content (147.02±3.28 mg/g). Among three different extracts, soluble free (Sol-F) extract
202 showed maximum phenolic content ranging from 141.08±2.34mg of GAE/g in *P.taeda* to
203 38.08±2.96 mg of GAE/g in *P.roxburghii* while water extract (Bound-W) showed minimum
204 phenolic content. Results were expressed as mean ± SD (n=3). Phenolic compounds are
205 reported to have positive correlation with the antioxidant activity due to their capability to act
206 as electron donors during free radical reactions¹⁶. In addition to genetic make up, it has been
207 observed that altitude and temperature are the major factors that affect phytochemical profile
208 of plants¹⁷. It was reported that the plant samples collected from lower temperature and

209 higher altitude sites showed higher concentration of phenolic content¹⁸. In the present work
210 various species of *Pinus* were collected from same location thus the probability of variations
211 that exist among them with respect to the contents of phenolics and flavonoid may be solely
212 due to genetic differences within them.

213 3.2 Total flavonoid content

214 Among three different extracts, soluble free (Sol-F) extract showed maximum flavonoids
215 while water extract (Bound-W) showed minimum phenolic content (Table 1 and Figure 3).
216 The flavonoid contents of the three fractions from the ten pine needle species are shown in
217 Table 2 & Figure 5. *P.taeda* had the maximum total flavonoid (Soluble-F + Bound-E +
218 Bound-W) content (21.91 ± 1.55 mg/g), followed by *P.echinata* (20.35 ± 0.84 mg/g), and
219 *P.patula* (19.53 ± 0.47 mg/g). Among selected species, the needles of *P.echinata* had the
220 maximum and *P.thunbergii* had the minimum ratio of total flavonoids to total phenolics.

221 3.3 Antioxidant activity

222 Antioxidant property in three extracts of pine needles belonging to ten species was estimated
223 using oxygen radical absorbance capacity (ORAC) of extracts. Among three different extracts,
224 soluble free (Sol-F) extract showed maximum antioxidant activity while water extract
225 (Bound-W) exhibited minimum anti-oxidant activity (Figure 4). Measurement of anti-oxidant
226 activity in three extracts revealed that *P. taeda* had the maximum Soluble-F antioxidant
227 activity (1151.7 ± 13.7 $\mu\text{mol Troloxg}^{-1}$), followed by *P. thunbergii* (1036.6 ± 20.7 μmol
228 Troloxg^{-1}), *P. patula* (913.4 ± 21.4 $\mu\text{mol Troloxg}^{-1}$), *P. merkusii* (849.8 ± 30.1 $\mu\text{mol Troloxg}^{-1}$),
229 *P. elliotii* (700.3 ± 12.7 $\mu\text{mol Troloxg}^{-1}$), *P. khasya* (659.3 ± 34.9 $\mu\text{mol Troloxg}^{-1}$), *P.echinata*
230 (629.9 ± 22.1 $\mu\text{mol Troloxg}^{-1}$), *P.greggii* (540.9 ± 17.1 $\mu\text{mol Troloxg}^{-1}$), *P.roxburghii* (419.2
231 ± 17.3 $\mu\text{mol Troloxg}^{-1}$) and *P.wallichiana* (406.6 ± 9.9 $\mu\text{mol Troloxg}^{-1}$). *P. khasya* had the
232 maximum Bound-E antioxidant activity (144.4 ± 8.3 $\mu\text{mol Troloxg}^{-1}$), followed by *P. patula*
233 (128.6 ± 6.4 $\mu\text{mol Troloxg}^{-1}$), *P. merkusii* (120.1 ± 3.7 $\mu\text{mol Troloxg}^{-1}$), *P. roxburghii*
234 (117.6 ± 6.8 $\mu\text{mol Troloxg}^{-1}$), *P. greggii* (79.9 ± 8.2 $\mu\text{mol Troloxg}^{-1}$), *P. wallichiana* (77.6 ± 6.2
235 $\mu\text{mol Troloxg}^{-1}$), *P. taeda* (66.8 ± 7.0 $\mu\text{mol Troloxg}^{-1}$), *P. elliotii* (66.3 ± 5.7 $\mu\text{mol Troloxg}^{-1}$), *P.*
236 *echinata* (54.4 ± 6.3 $\mu\text{mol Troloxg}^{-1}$) and *P. thunbergii* (53.7 ± 9.1 $\mu\text{mol Troloxg}^{-1}$). *P. khasya*
237 had the maximum Bound-W antioxidant activity (95.1 ± 8.5 $\mu\text{mol Troloxg}^{-1}$), followed by
238 *P.taeda* (90.1 ± 6 $\mu\text{mol Troloxg}^{-1}$), *P.patula* (90.0 ± 7.9 $\mu\text{mol Troloxg}^{-1}$), *P. wallichiana*
239 (66.2 ± 4.8 $\mu\text{mol Troloxg}^{-1}$), *P. merkusii* (56.9 ± 1.2 $\mu\text{mol Troloxg}^{-1}$), *P.greggii* (49.4 ± 4.9 μmol
240 Troloxg^{-1}), *P.roxburghii* (39.9 ± 2.9 $\mu\text{mol Troloxg}^{-1}$), *P. echinata* (32.4 ± 1.2 $\mu\text{mol Troloxg}^{-1}$), *P.*
241 *elliotii* (26.2 ± 3.2 $\mu\text{mol Troloxg}^{-1}$), and *P. thunbergii* (24.4 ± 2.1 $\mu\text{mol Troloxg}^{-1}$). The

242 antioxidant capacities of the Soluble-F fractions of the ten pine needles were higher than
243 those of Bound-E and Bound-W fractions of needles. *P. taeda* had the highest total
244 antioxidant activity ($1308.6 \pm 26.8 \mu\text{mol Trolox}^{-1}$), followed by *P. patula* ($1162.0 \pm 35.7 \mu\text{mol}$
245 Troloxg^{-1}), *P. thunbergii* ($1114.7 \pm 33.1 \mu\text{mol Troloxg}^{-1}$), *P. merkusii* ($1026.8 \pm 35.1 \mu\text{mol}$
246 Trolox^{-1}), *P. khasya* ($898.8 \pm 51.7 \mu\text{mol Troloxg}^{-1}$), *P. elliotii* ($792.8 \pm 21.6 \mu\text{mol Troloxg}^{-1}$),
247 *P.greggii* ($670.3 \pm 30.2 \mu\text{mol Troloxg}^{-1}$), *P.roxburghii* ($576.7 \pm 26.9 \mu\text{mol Troloxg}^{-1}$) and *P.*
248 *echinata* $553.5 \pm 20.9 \mu\text{mol Trolox/g}$. The results show a direct correlation between phenolic
249 concentration and anti-oxidant potential of species ($R^2=0.847$). This relationship has also
250 been established in case of *P. nigra* needles using correlation analysis where a significant
251 linear correlation between the antioxidant activity and total phenol, flavonoid and tannin
252 contents was reported in the case of *salazmani*, *pallasiana* and *nigra* subspecies. However, no
253 significant relationship between antioxidant activity and total phenols, flavonoids and tannin
254 was recorded for *laricio* subspecies¹⁹. Needles obtained from *Pinus* have been traditionally
255 used to prepare drinks and as a medicine for several centuries in South-eastern Asian
256 countries specially in China²⁰, thus the results related to antioxidant potential of these plants
257 in this section indicated pines as a promising source of natural antioxidant foods.

258 3.4 Antimicrobial activity

259 Antibacterial and antifungal activities of the different extracts obtained from ten species of
260 *Pinus* were evaluated using the disk-diffusion method, and the results are presented in [Table](#)
261 [3](#). *Pseudomonas aeruginosa* was resistant to each of three extracts of ten pine needles while
262 other gram negative bacteria showed greater degree of resistance in comparison to gram
263 positive bacterial strains. This resistance can be attributed to the complexity of their double
264 layer cell membrane of gram negative bacterial strains in comparison with the single
265 membrane of Gram positive bacteria²¹. For fungal strains, *P.khasya*, *P.taeda* and *P.thunbergii*
266 showed growth inhibitory activity in each of three extracts against *C.albicans*. Overall, lower
267 MIC ([Table 2](#) and [Figure 5](#)) of extracts were needed to inhibit *S.typhimurium* ($<10.0 \text{ mg}$
268 mL^{-1}). The highest MIC was obtained with the Bound-W extract of *P.merkusii* (36.1 mg
269 mL^{-1}) against *S.schenckii*. In contrast, lowest MIC was obtained with Sol-F extract of
270 *P.taeda* (3.0 mg mL^{-1}). In this study, Soluble-F (soluble free) fraction revealed higher
271 antibacterial activities in comparison to Bound-W and Bound-E indicating that most of the
272 antibacterial constituents are extracted with soluble free fraction while nearly every fraction
273 in one species or another revealed growth inhibitory impact on selected fungal strains
274 indicating presence of antifungal constituents in each fraction.

275 **4. Conclusion**

276 Our research furnishes the presence of several phenolics and flavonoids components and
277 related anti-oxidant and antimicrobial properties in native and cultivated species of *Pinus*
278 growing in Indian Himalayas. The antioxidant and antimicrobial activities reported in the
279 current research confirms that the therapeutic value and biological efficacy of secondary
280 metabolites in these species is very high and can lead to the development of novel, safe, and
281 cost effective drugs in future. However need of hour is to conduct detailed and elaborative
282 research on ethnobotanical and pharmacological investigations with simultaneous public
283 awareness for the best utilization of medicinal and other beneficial properties of pines.
284 Involvement of industrial entrepreneurs should also be promoted so that they can come
285 forward with novel concepts and desired technologies and move towards sustainable use of
286 pines. However, ecotoxicological implications must be imperative prior to marketization.

287 **Conflicts of interest**

288 All contributing authors declare no conflicts of interest.

289 **Acknowledgement**

290 Author is thankful to Head, department of Botany, Lucknow University for providing
291 necessary research facilities. We also confirm that the authors have no conflict of interest.

292 **Authors Contribution**

293 Formal analysis, Lav Singh and Praveen Chandra Verma; Investigation, LAV SINGH and
294 Praveen Chandra Verma; Resources, Pooja Dixit, Atul Kumar Upadhyay, Ravi Prakash
295 Srivastava and Shiva raman Pandey; Supervision, Gauri Saxena; Validation, Gauri Saxena;
296 Writing – original draft, Lav Singh

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363 Sussex, England.

Table 1: Total phenolic and flavonoid contents in selected pine spp. (Soluble-F= soluble free, Bound-E= bound ethyl acetate and Bound-W= bound water) collected from Indian Himalayas.

<i>Pinus</i> spp	Soluble-F(mg/g)		Bound-E (mg/g)		Bound-W (mg/g)		Total (mg/g)	
	Phenolic content	Flavonoid content						
<i>P.merkusii</i>	85.91±3.23	11.41±0.36	3.91±0.22	3.52±19	2.08±0.06	0.92±0.01	91.90±1.87	15.85±0.25
<i>P.khasya</i>	67.1±1.03	12.94±0.12	4.87±0.22	4.66±0.26	2.3±0.12	0.88±0.03	73.88±1.37	18.48±0.41
<i>P.taeda</i>	141.08±2.34	16.46±1.23	2.85±0.17	3.48±0.26	3.09±0.07	1.97±0.06	147.02±3.28	21.91±1.55
<i>P.elliottii</i>	91.51±1.200	9.81±0.26	3.04±0.24	3.25±0.08	2.54±0.17	1.48±0.12	97.09±1.61	14.54±0.46
<i>P.echinata</i>	61.84±2.16	14.97±0.56	1.72±0.09	3.83±0.15	2.04±0.02	1.55±0.13	65.60±2.27	20.35±0.84
<i>P.thunbergii</i>	139.67±0.89	13.09±0.45	2.14±0.14	3.62±0.12	1.83±0.05	1.8±0.05	143.64±1.08	18.51±0.62
<i>P.patula</i>	102.41±2.40	13.71±0.25	3.99±0.32	4.89±0.19	1.66±0.13	0.93±0.03	108.08±2.85	19.53±0.47
<i>P.greggii</i>	51.72±3.38	9.87±0.46	3.77±0.69	3.51±0.11	3.62±0.23	1.44±0.21	59.11±4.3	14.82±0.78
<i>P.wallichiana</i>	40.27±1.34	8.41±0.15	3.77±0.69	2.51±0.04	3.02.1±0.07	0.94±0.17	47.06±2.1	11.86±0.36
<i>P.roxburghii</i>	38.08±2.96	7.78±0.22	3.93± 0.21	2.51±0.04	1.25±0.05	0.68±0.07	43.26±3.22	10.97±0.33

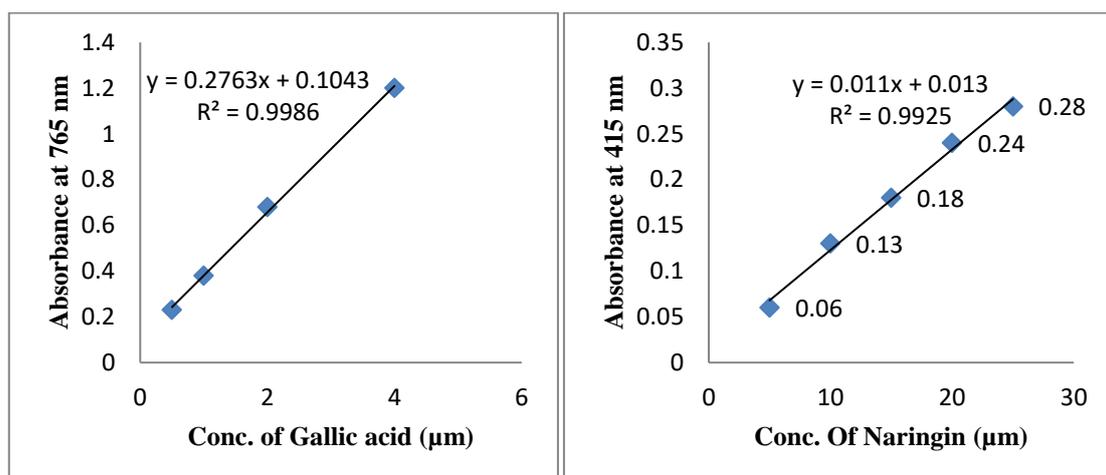


Figure 1: Standard curve with gallic acid of concentration in ten species of *Pinus* ranging from 0.5µg/ml to 4µg/ml. and with Naringin of concentration ranging from 5µg to 25µg in the reaction mixture.

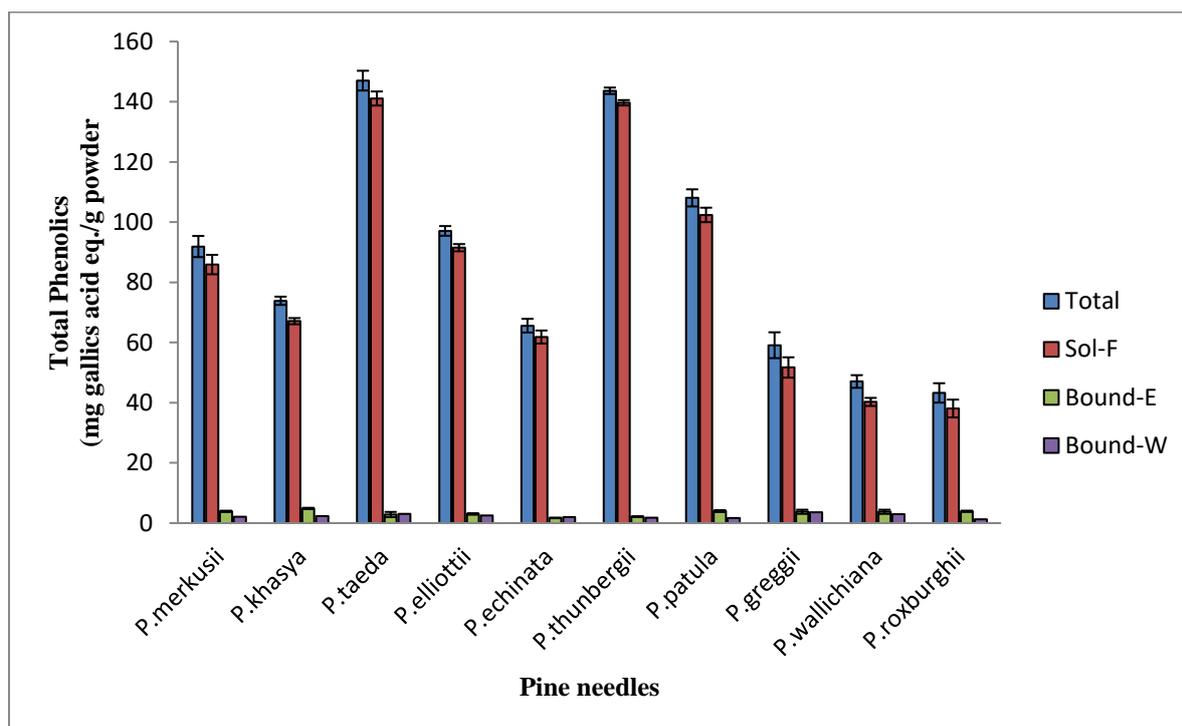


Figure 2: Total phenolics in soluble free (Soluble-F), bound ethyl acetate (Bound-E), and bound water (Bound-W) fractions needles of various pine species under study. Values are mean±SD of triplicate measurements.

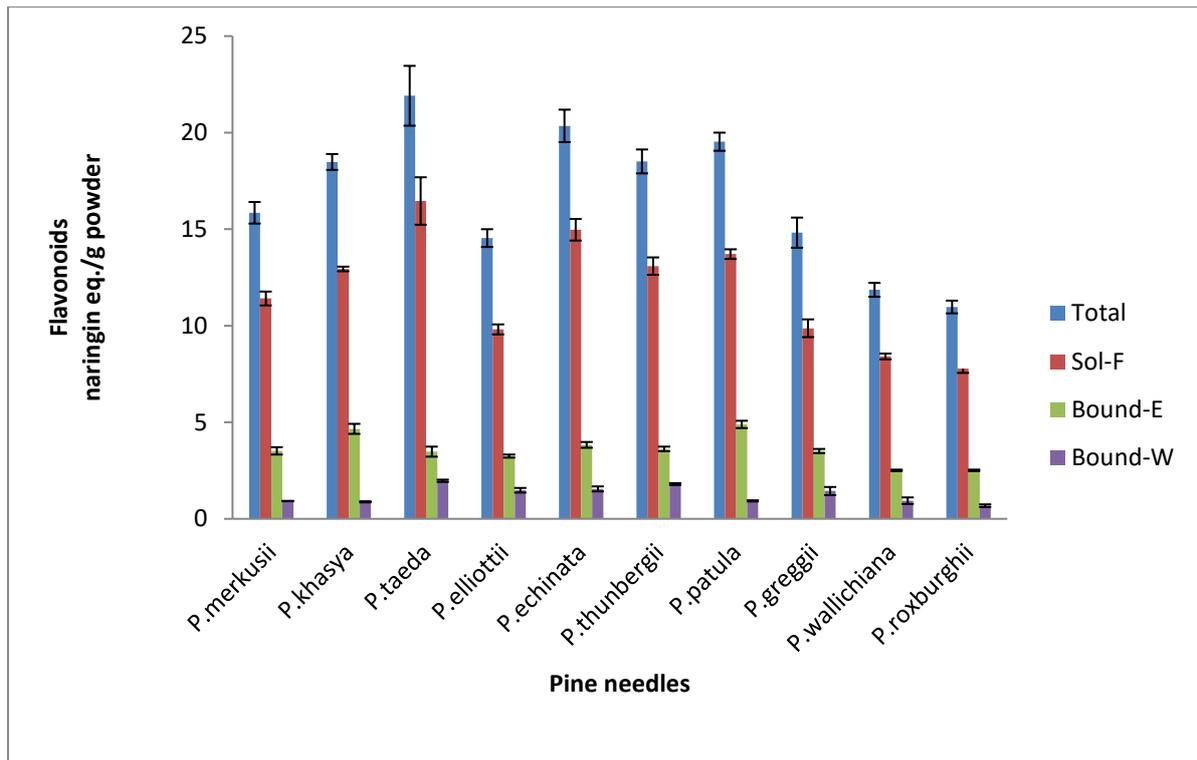


Figure 3: Total Flavonoids in soluble free (Soluble-F), bound ethyl acetate (Bound-E), and bound water (Bound-W) fractions. Values are mean \pm SD of triplicate measurements.

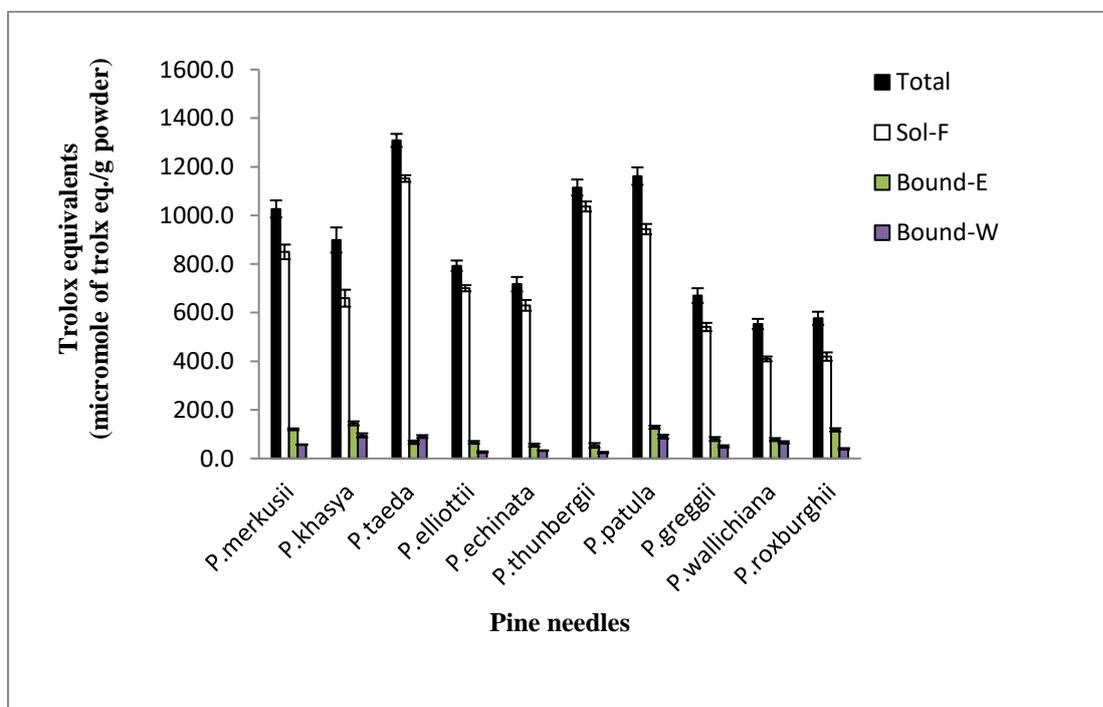


Figure 4: Anti-oxidant activity in soluble free (Soluble-F), bound ethyl acetate (Bound-E), and bound water (Bound-W) fractions. Values are mean \pm SD of triplicate measurements.

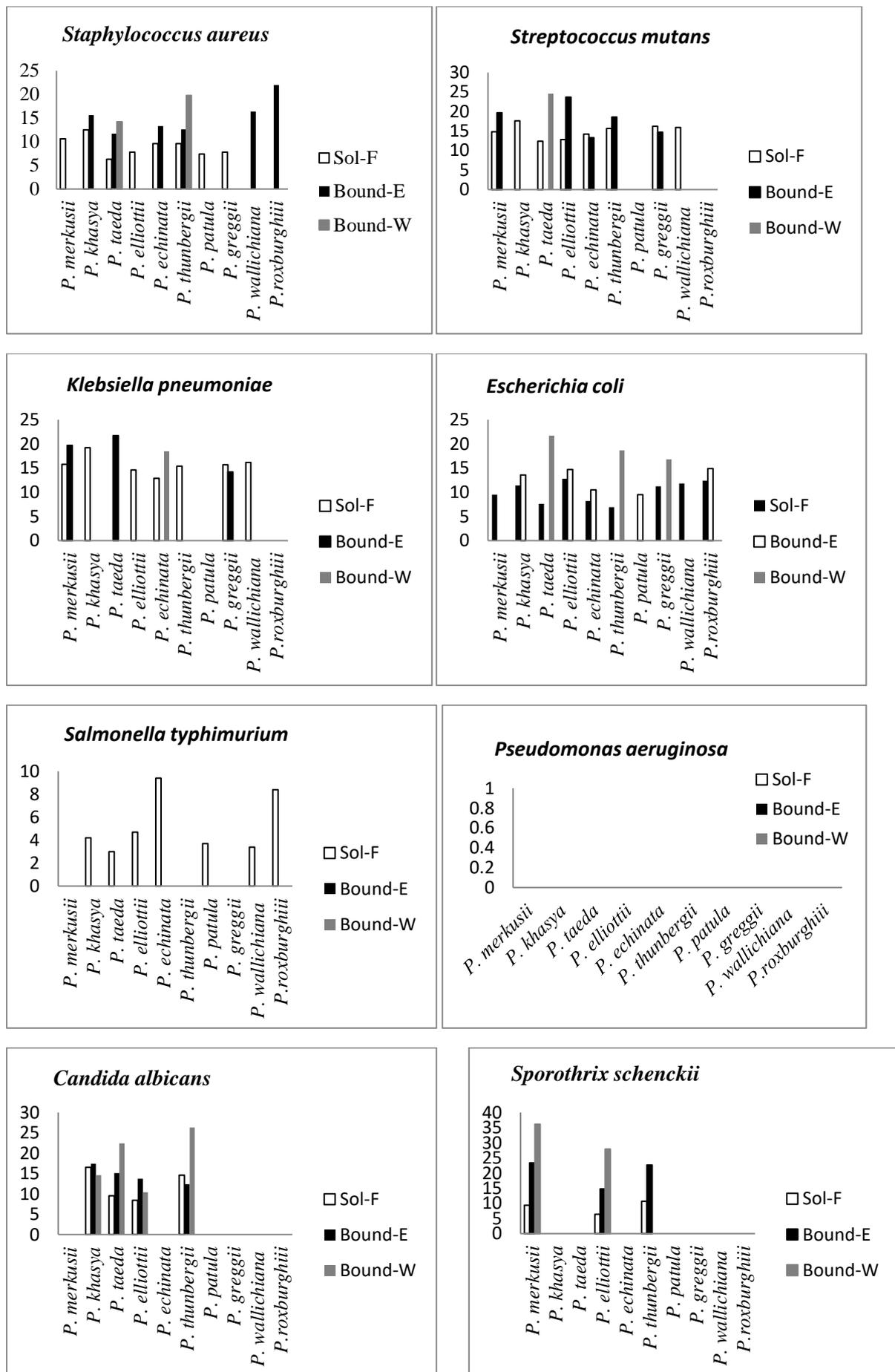


Figure 5. Minimum inhibitory concentration (MIC) of three different extracts of ten *Pinus* species against six bacterial and two fungal strains. Values are expressed in mg.mL^{-1} .

