UTILIZATION OF WILD PLANTS SPECIES FOR INNOVATIVE BIO BASED INDUSTRIAL COMPOUNDS

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ABSTRACT

Pinus Roxburghii (pine) and similar others species of this family are very important and dominated trees of forest from North east of Punjab Province (Murree and Kotli sattian). The needles leaves, stem, resins and cones of these trees are important due to their domestic and commercial uses like food , cosmetics and fertilizers . In the current study *Pinus* needles leaves and resins were chemically analyzed to assess levels of phenols, flavonoids, tannins and total oil contents by using different analytical methods as well as GC-MS and FT-IR techniques. Antioxidants, Anti mycobacterium tuberculosis (MT) and cytotoxicity assays were performed to determined bioactivities of different extracts of leaves. Where as total oil contents of resins were determined by using Soxhlet method and oil was converted into different products like glycerin and biodiesel by using trans etherification process. Results indicates that *pinus* leaves contains total phenols (7.26±1.52) flavonoids (14.53±2.45) and tannins (4.36±1.23). Where as total oils was found in *pinus* needles leaves (1.92 ± 0.28) as compared to pinus resins . GC-MS analysis exposed higher quantity of organic compounds (fatty acids) , those were further confirmed by indication of their functional group by FT-IR analysis. According to results methanolic extracts of Pinus roxburgii has provided higher antioxidant values (DPPH, IC_{50} = 38.36±4.58 µg/ml) and higher zones of inhibition for MT strains as compared to lower value of brine shrimp cytotoxicity assay and these values are probably due to presence of total phenols , flavonoids and essential oils contents

Key words: *Pinus* needles leaves, Resins, Organic acids, Bioactivities

INTRODUCTION

Especially for small-scale producers, agro forestry may be a valuable source of low-cost revenue that applies to many types of integrated land. It also helps promote long-term, sustainable, and renewable forest management, which contributes to a green economy (Beech et al., 2017).

Forests in Pakistan, particularly in the northeastern regions of Punjab and elsewhere, are home to trees belonging to the Pinaceae family. The chir pine, or Pinus roxburghii, has a long history of economic usage due to its many useful parts, including its leaves, stem, resins, and cone (Keeley, 2012).

Due to their shoot dimorphism, which comprises small shoots (fascicles) surrounded by bud scales at the base and one to eight slender needles, Pinus is a member of the Pinaceae family. Pinus trees are easily recognizable by their robust, woody cone scales, which reveal the apical structure both in the mature cone and after the first growth season, or bump. It is now believed that Pinus is a single taxon (Hussain et al., 2008). Each needle in the pine subgenus contains two pulvini, or fibrovascular bundles, that diverge at the bases of the cataphylls and typically have persistent sheaths. Per fascicle, you'll find anywhere from two to eight needles; the resin canals may be found in a variety of positions, including septa, internal, and medial external; and the seed wings can be either articulated or repressed (Briskin, 2000).

Pinus trees or bushes, which include many of the most notable conifers in terms of commercial importance, such as cedars, firs, hemlocks, larches, pines, and spruces.Use the inner bark of a pine tree to bandage wounds and scrapes without worrying about spreading germs. Channel tape, a cloth, or cordage are used to apply it to wounds. According to Hussain et al. (2008) and others

Long, narrow leaves that look like needles characterize needle leaf trees, which are evergreen conifers. The tree's leaves don't fall off at any point throughout the year; rather, they gradually and steadily replace them. According to Keeley (2012) and riskin (2000), the narrower needles of a tree are more windproof and watertight than the broader, bigger leaves of a broadleaf or deciduous tree. There are around 114 species in the Pinus genus that produce needles; these evergreen trees may be found all over the globe. According to Dynesius and Janssor (2000), the Pinaceae family is comprised mostly of the subgenera Pinus and Strobus, which are the most prevalent types of pine trees.

Ever since ancient times, pinus needles have been used for a variety of purposes, including animal care, roofing, and fertilizer. Some cosmetic and related compounds have been derived from leaves, and they have also been used as feed and food (Faster and Duke, 2000).

The oleo-resin (crude turpentine) released by different pine tree species is the source of rosin, a solid resin. The process of rosin extraction involves bending the tree's stem. According to Ahmad et al. (2014), natural resins are organic compounds that are usually combustible, translucent, or fusible, and may range in color from yellowish brown to opaque white.

Alternative methods of extraction include using a naphtha solvent procedure or heating the oleo-resin to evaporate the essential oils (spirit of turpentine) (Roy et al., 2004). When making varnishes, rosin is often mixed with other resins.

The conifer cone is a structure found on gymnosperm plants that contains seeds. The fruit is typically spherical or oval in shape and covered with scales and bracts that form an axis. In addition to being tasty, cone seeds are rich in minerals, including essential oil (Gernandt, 2005).

Important domestic uses for Pinus roxburghii wood include firewood, household products, furniture preparation, and more. If you're experiencing flu-like symptoms, drinking some pine needle tea will help clear your system of harmful substances. Essential oils have a long history of medical, cosmetic, antiparasitic, fungicidal, bactericidal, and virucidal usage. These days, you may find them in a lot of different places, including the culinary, cosmetic, agricultural, and pharmaceutical sectors (Richandson, 2000).

For the treatment of various human ailments, almost 80% of the global population relies on home made remedies. A large variety of modern drugs, including dioxin, morphine, codeine, ibuprofen, vinblastine, cocaine, emetine, ephedrine, vinocristine, pilocarpine, and many more, have their origins in medicinal plants (Farjon, 2018). The pharmaceutical industry's interest in medicinal plants rekindled in the second half of the twentieth century, when manufactured science made more strides than in the first, and they looked to natural plant sources as synthetic platforms for drug amalgamation. The naturally occurring chemicals derived from medicinal plants are safer and may one day replace the synthetic pharmaceuticals that make up around 70% of our current supply.

medicines (Masango, 2005; Nergiz and Donmez, 2004).Antioxidant chemicals, rather than vitamins and carotenoids, are found in abundance in many therapeutic plants. Plants include medicinal compounds that have great biological importance and activity. The antioxidant activity and phenolic and secondary metabolite content of therapeutic herbs were higher than that of commonly consumed fruits and vegetables, which are known to be excellent sources of antioxidants in the diet (Angel et al. 2019; Fisher, 1991).

Also tested was a canella winterana essential oil fraction. The compounds were tested for their antimycobacterial properties against Mycobacterium tuberculosis, Mycobacterium avium, and Mycobacterium kansasaii using the Bactec 460-TB radiometric system, Middlebrook 7H11 agar medium, and assurance of bacterial appropriate counts. Miranda et al. (2012) found that three mixtures—ibogaine, voacangine, and texalin—had antimycobacterium activity.

Origanumdayi post (Labiatae), Artemesiamonosperma (Asteraceae), and Urticamembranacea (Urticaceae) were the plants used to extract the compounds. In several human inferred tumor cell lines and crucial societies established from patients' biopsies, each of the three plant extracts demonstrated dosage- and time-subordinate killing properties. Since the plant extracts had no effect on the fundamental cellular structures of solid human cells, the activity was limited to tumor cells (Jelonek et al., 2016).

So far, many methods have been used to extract essential oil from plant matter, such as steam refining, soluble extraction, and others. Two significant categories may be drawn from the basic oil extraction method according to the temperature that is used for extraction: extraction at low or high temperature and room temperature. According to Montalouti et al. (2010), the methods include volatile headspace collection, hydro distillation, steam distillation, soxhlet extraction, and water and steam refining. According to Desouza et al. (2007), these natural products have bioactivity as antibacterial, antiviral, antioxidant, and anti-diabetic agents, and they may play a role in the prevention and treatment of cancer and cardiovascular diseases like atherosclerosis and thrombosis.

MATERIAL AND METHODS

Collection and preparation of samples

In little plastic bags that were clearly marked with the date and locations of collection, we gathered samples of pinus Roxburghii (chir pine) leaves and resins. Gathering samples based on traditional medicinal applications by rural Murree (Rawalpindi) residents. The resin was carefully collected from the pinus tree bark and stored in little plastic bags for further processing. A botanist from the Department of Botany at PMAS Arid Agriculture University Rawalpindi confirmed the samples' identities, and they lodged voucher specimen (No. 156). The UIBB, PMAS Arid Agriculture University Rawalpindi received around 3 kg of plant material for further processing (Nergiz and Donmez, 2004).

The bioactivities of different phytochemicals and oils were identified by extracting them from pinus needles leaves and resins (Newman et al., 2000).

To remove dust and other unwanted particles, plant materials were rinsed with demineralized water. A combination of shade and sun drying, followed by overnight oven drying at 50 °C, was used to prepare the leaf samples. We used an electric grinder and an 80-mesh sieve to crush the dry samples, and then we stored them in plastic bags at a lower temperature until we needed them again. Oil was extracted from samples of pinus resins after they were washed and dried in the sun (Cai et al., 2004).

Extraction of Pine Resins from Pine Tree Bark

Making a small incision deep enough into the tree's trunk to pierce the vacuoles allows sap to flow out (a process called tapping), and then, after a few days, the tree will heal itself by sealing the wound with resin (Kohler et al., 2019). Translucent or translucent, with a color range from yellowish to brown, natural resins are organic compounds that are usually combustible and fusible. As a general approach, one might use alcoholic solvents to extract the medicine, and then, after adding a concentrated alcoholic extract to a significant amount of water, resin could be precipitated. To isolate volatile oils from resin, one may use hydrodistillation or distillation as a technique (Kohler et al., 2019; Cai et al., 2004).

Determination of total phenols:

Using a technique described by many authors (Newman et al., 2000), the content of phenol in the leaves and resins was measured in various solvents. In brief, 0.5 ml of Folin-Ciocalteu reagent was applied to 100 µl of extract after diluting it with 3 ml of distilled water. After waiting three minutes, 2 milliliters of 20% sodium carbonate were added and everything was mixed well. Using a spectrophotometer (a Shimadzu UV-1800), the color was generated and the absorbance was measured at 650 nm. The standard was gallic acid, and the standard curve was produced using various amounts of gallic acid (R2 = 0.9926). Gallic acid equivalents (GAE) mg/100 g of dry matter were used to represent the quantities of total phenolics (Monfalouti et al., 2010).

The Determination of Flavonoids

The technique described by Husain et al. (2008) was used to determine the flavonoid contents of the extracts. In a nutshell, 4 milliliters of distilled water and 0.3 milligrams of quercetin were put to a 10-milliliter flask along with a 1-milliliter portion of the extract or a standard solution of the compound.

One milliliter of sodium binitrotol. The following steps were taken after 5 minutes: 0.3 ml of 10% $AlCl_3$ was added, 2 ml of 1M NaOH was added after 6 minutes, and finally, 10 ml of distilled water was added to increase the volume. According to spectrophotometer readings taken at 510 nm, flavonoid contents were reported as milligrams of quercetin equivalent per gram of material (Kharzer et al., 2019; Burt, 2004).

Estimation of Tannins:

The Folin Denis technique was used to estimate the total tannin content in the leaves extract. The blue hue, produced when tannins reduce phosphotungstomolybidic acid, is quantified by this method. A colorometric analysis of tannins was performed in accordance with the procedure by combining 1.0 ml of extract with a standard solution of tannic acid and 7.5 ml of distilled water. After that, 0.5 milliliters of Folin Denis reagent was added, and 1 milliliter of sodium carbonate was added. The absorbance was measured at 700 nm after diluting the solution with 10 ml of distilled water. According to Cushnie and Lamb (2011), the total tannic acid content was given as milligrams of tannic acid equivalent per gram of extract.

Extraction of oils by Soxhlet method

Oil was extracted from *pinus* needles leaves and *pinus* resins by using Soxhlet apparatus as reported (Alanis et al., 2003; Essawi Srour, 2000). Total 15 grams of a powdered form of a sample was taken in the clean thimble. Then fix the thimble in the loading chamber and connect the loading chamber with the condenser

. Added methanol (300 mL) in the flask and connect it to a continuous supply of water. Fix the whole assembly in the heating mental and heat the flask up to 50 ºC. High temperature caused the vaporization of the solvent and vapors moved upward by the tubes and hit the condenser and turned to hot liquid (Muthu et al., 2006).

Conversion of oil into Fuel (Biodiesel) and glycerin

Crude oil contains many impurities such as free fatty acids, phospholipids and sterols whereas refined oil contains the very small amount of free fatty acids and other impurities. These impurities in the form of FFA and water have significant effects on the reaction, glycerides.

Alcoholysis is the chemical reaction in which the oil or fat reacted to the methanol followed by alkali (sodium hydroxide) catalyst to produce ester and glycerol. For getting the high yields of ester in transesterification reaction, use of an extra quantity of methanol is preferred (Alanis et al., 2003).

Trans esterification process

In the base catalyzed reaction the methanol as a solvent and alkali (sodium hydroxide) was used as a catalyst. Different catalyst concentrations of NaOH such as 0.5 %, 1 %, or 1.5 % (w/w) may be used along with the methanol. The homogeneous solution of sodium hydroxide and methanol was prepared. Oil has firstly heated to 60 ºC and then cooled to room temperature. Then add the catalyst, and methanol mixture in the oil flask, set the whole in the reaction assembly apparatus, the mixture was stirred at 800 rpm for 2 h. After the complete reaction time, the whole mixture was taken in separating funnel with the passage of time, two layers were separated. The upper layer was methylated ester, and the lower layer was glycerin. The upper layer consists of different impurities such as unreacted methanol, catalyst, water, and glycerin . Further more neutralization and washing process were carried out for refinement of Biodiesel (Al-Snafi, 2003).

Physical and chemical analysis of Fuel (Biodiesel)

Different Physical and Chemical analysis were carried out for assessment of quality of Biodiesel like pH, Iodine , acid and saponification values by following AOAC method (Muthu et al., 2006; Al-Snafi, 2013).
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Analysis of oil for fatty acids with GC-MS

The methyl esters (FAME) were determined by transmethylating 0.20 g of pinus needles leaves oil in 2 mL of n-heptane with a cold solution of KOH (2 mol L−1) (200 μL). Using Gas Chromatography-Mass Spectrometry, the extracted oil's composition was investigated. In order to examine the sample, the HP framework was used. A HP 5973 Mass spectrometer (MS; Agilent Advances Inc. USA) was attached to the 6890N along with a DB-5 section (30 m length, 0.25mm inner distance across, and 0.25 µm stationary stage film thickness). At 235 °C, the injector was turned on. Based on the following parameters, the GC stove temperature was adjusted as follows: the broiler's base temperature was maintained at 40 °C for 2 minutes, then it was heated to 450 °C at a rate of 4 °C per minute, and then it was left at that temperature for 8 minutes. An absolutely pure helium gas stream of 1 mL/min was used as a portable stage. A weaker mixture of base oil was introduced.

to a volume of 1 μL in the split-less mode. At an ionization energy of 70 eV, an electron ionization framework was used to generate mass spectra. The mass spectrometer's particle source temperature was set at 180°C, and the dissolvable deferral was set at 5 minutes. Between thirty and forty amu, the mass spectra filter expands. Without using remedy factors, the rate structure of an example was registered using GC top territories (Cushine and Lamb, 2011; Feng et al., 2011). Mass spectra comparisons between a molecule and the NIST-2008 MS library were the first step in completing the differentiating evidence of essential oil components. Step two included using the same GC-MS settings as the basic oils to resolve the maintenance lists of the separated mixtures with regard to the maintenance periods of the standard C9–C24 n-alkanes. We compared the disseminated information for the guarantee of elution requests and differentiating proof of mixes with the figured maintenance lists of mixes. Finally, co-infusion served as the differentiating evidence of concoction.

Bioactivity assessment of *Pinus* needles leaves

Antioxidant activity

Following a previously described methodology (Feng et al., 2011), the antioxidant activity of plant extracts was assessed. Consequently, the following bioassays were used to determine the antioxidant content.

The DPPH Scavenging Bioassay

The DPPH scavenging activity was measured using a modified version of the technique described by Muthu et al. (2006). Antioxidants' capacity to neutralize the 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical provided the basis for this technique. A falcon tube was used to collect 100 μL of the sample solution, which was then combined with 4mL of DPPH solution (0.1 mM) and mixed violently. After that, the mixture was left to incubate at room temperature for 30 minutes in the dark, covered with aluminum foil to prevent heat exposure. The solution's absorbance at 517 nm was measured using a UV-Vis spectrophotometer. The equations used to determine percentage inhibition are as follows:

$$\% \text{ Inhibition} = A_{blank} – A_{sample} x\ 100 / A_{blank}$$

ABTS scavenging bioassay

Feng et al. (2011) previously described the procedure that was used to conduct the ABTS radical scavenging bioassay. To make the ABTS radical cation, 5 mL of a 7 mM aqueous ABTS solution was mixed with about 88µL of 140 mM potassium per sulphate, bringing the final solution concentration to 0.7cm-1. The amount of ethanol needed to make the leaf extracts was 25 µL. The final percentage decrease in absorbance was measured at 730nm after adding 10µL of the sample solution to the reaction mixture. Based on the initial absorbance of the reaction mixture, it was estimated that the end absorbance would be 20-80% lower.

The Scavenging of H2O2.

Using the approach described, the scavenging of H2O2 was measured. A solution of 0.6 mL H2O2 containing about 4 mM was added to 4 mL of extract and left to incubate for 10 minutes. Using a spectrophotometer, we determined the solution's absorbance at 230 nm in comparison to a blank solution. Potency of free radical scavenging as measured by the percentage of H2O2. The reduced H2O2 value demonstrated that the leaf extracts had a good ability to scavenge free radicals (Edelman et al., 2016).

A statistical analysis

Results were presented as means, standard deviations, and percentages after statistical analysis using one way ANOVA on the collected data..

RESULTS AND DISCUSSION

Samples of *pinus* needles leaves and *pinus* resins were chemically analyzed for various parameters and results were mentioned in following sections

Assessment of oil contents of needles leaves and resins

The extraction of oil was carried by distillation method by using Soxhlet apparatus and oils was extract from needles of *Pinus Roxburghii*, and *pinus* resins and results were mentioned [Tables 1-2].

Table 1. Extraction of oils (%) from Needle leaves samples of *pinus* roxburgii

Samples	Methanol	Ethanol	Acetone	Hexane
Needle leaves 1	45.5 ± 1.2	41.5 ± 1.4	11.6 ± 0.9	8.6 ± 0.6
Needle leaves 2	42.4 ± 1.5	37.6 ± 1.2	3.2 ± 2.1	4.7 ± 0.8
Needle leaves 3	33.5 ± 2.3	31.4 ± 0.7	1.6 ± 1.4	1.91 ± 0.7

Mean ± S.D (n=3).

Table 2. Extraction of oils (%) from resins samples obtained from stems of *pinus* Roxburgii.

Samples	**Methanol**	**Ethanol**	**Acetone**	**Hexane**
Resins 1	51.5 ± 1.5	48.3 ± 1.8	14.5 ± 0.8	12.7 ± 0.7
Resins 2	45.6 ± 1.1	41.5 ± 1.1	6.5 ± 2.3	3.9 ± 0.9
Resins 3	36.4 ± 2.1	31.0 ± 0.9	2.7 ± 1.1	1.90 ± 0.6

Mean ± S.D (n=3).

Table 3. Extraction of Biodiesel and glycerin after trans esterification process

Samples	**Biodiesel (%)**	**Glycerin (%)**
Methanol	10.5 ± 1.2	15.6 ± 3.2
Ethanol	8.2 ± 1.6	11.3 ± 1.5
Acetone	6.3 ± 1.1	7.2 ± 1.5
Hexane	7.4 ± 1.6	9.3± 1.6

Mean ± S.D (n=3).

Conversion of oil into Biodiesel and glycerin

Results regarding biodiesel and glycerin production, after trans esterification process of crude oil are given into table3. Higher quantity of Biodiesel was produced when transesterification was carried out by using methanol and sodium hydroxide, 10.5 ± 1.2 % / 100 ml of crude oil . Where as 15.6 ± 3.2 % of glycerin was produced when 100 ml of crude oil was used for transesterification process .

Pure glycerin / glycerol has various uses in food, pharmaceutical , medical and personal care industries as reported by different research workers (Graf et al., 2010; Edelman et al., 2016).

Physical and chemical analysis of Biodiesel

Conformity tests of Biodiesel were carried out and results of pH, acidity, iodine values and saponification values are given in table 4

Table 4 . Various parameters of Biodiesel

Parameter	Results
Color	Pale yellow
pH	6.5
Acid value (mg of NaOH/g of oil	1.3 ± 0.01
Iodine (mg of I2/g of oil)	81.5± 0.2
Saponification value (mg of KOH/g of Fat	175± 1.18
Refractive index	1.46± 0.01

Mean ± SD

Analysis of Phyto chemicals

Quantitative analysis of needle leaves and resins are given in table 3. According to results higher quantity of flavonoids fallowed by total phenols and tannins were present in needles leaves. Where as lower quantity was obtained from pinus resins (Roy et al., 2004).

Table 5. Analysis of methanolic extracts of *pinus* needle leaves and resins of *Pinus Roxburghii* (chir pine) for different phytochemicals

Constituents	**Resins**	**Needles leaves**
Total phenol mg/g	3.28±0.72	7.26±1.52
Total flavonoids mg/g	4.15±1.38	14.53±2.45
Total tannins	2.87±0.54	4.36±1.23

Mean ±SD (n=3)

Analysis of oil by GC- MS

Oil extracted from various samples of pinus needles leaves and resins was analyzed by GC – MS .It was found many fatty acids were present [Table 5].

Table 6 Fatty acid contents of oil from *pinus* resins analyzed by GC-MS

Name	**RT**	**%compounds**
1-DODECANOL, 2-OCTYL-	8.801	0.008304
TETRAPENTACONTANE, 1,54-DIBROM	11.042	53.84524
1(2H)-NAPHTHALENONE, 6-(1,1-DI	21.181	41.54379
1-DODECANOL, 2-HEXYL-	32.356	0.013741

There were four compounds of fatty acids detected from oil of by GC-MS . However, two compounds like tetrapentacontane (53.84 %) and Napthahalenone (41.5%) were present with higher concentration (Tables 6 and7).

Table 7 Fatty acid contents of oil from needles leaves analyzed by GC-MS

Name	RT	Area	%composition
OCTADECANE, 1-CHLORO-	22.422	11131664384	96.51861
1-DODECANOL, 2-OCTYL-	33.801	25381554	0.220074
1-DODECANOL, 2-HEXYL-	34.807	6787248.5	0.05885
HEPTACOSANE, 1-CHLORO-	36.943	10392788	0.090112
17-PENTATRIACONTENE	37.098	3289870.25	0.028525
1-PENTACONTANOL	37.538	744749.688	0.006457
TETRAPENTACONTANE, 1,54-DIBROM	42.08	53473556	0.46365

There were seven compounds of fatty acids detected from oil of pinusroxburgii by GC-MS . However, compound like OCTADECANE, 1-CHLORO- (96.51) is present with higher concentration (Tables 6-8)

Table 8. Number of Carbon atoms of fatty acid found in organic compounds

Fatty acid	**P. R (%)**	**C**
C18 :O	96.5	0.08
C 15: 0	0.64	
C 20 :0	0.22	53.84
C 27:0	0.31	0.13
C35: 0	0.62	-
C 54: 0	0.46	-
C8 :0	-	41.54

Bioactivities of *pinus* needles leaves

Antioxidant activities of leaves extract

The DPPH, ABTS, and H2O2-scavenging bioassays were used to determine the antioxidant properties of the Pinus needle extracts. The needles' mechanism of scavenging Tables 9–10 show that the H2O2 scavenging test yielded much higher results than the ABTS and DPPH scavenging assays.

Table 9 . Antioxidant activity of leaves extracts of *Pinus Roxburghii* (chir pine) *(*IC50 values µg/ml)

Extract 100 µg/ml	**DPPH**	**H_2O_2**	**ABTS**
Ethanol	45.17±3.26	58.54±5.26	45.32±2.81
Methanol	25.38±4.15	42.56±3.15	39.46±2.28
N hexane	52.18±1.36	61.52±4.85	48.24±1.35
Ascorbic acid	9 .65±2.52	7.62 ±1.36	16.25±2.38
Gallic acid	6.34±1.32	5.65±1.25	8.26±1.36

Means ± SD, (n = 3).

Table 10. Antioxidant activity of *Pinus resins* extracts *(*IC50 values µg/ml)

Extract 100 µg/ml	**DPPH**	**H_2O_2**	**ABTS**
Ethanol	36.17±1.25	45.69±4.15	39.45±2.16
Methanol	27.28±1.48	38.21±3.17	32.89±4.36
N hexane	45.16±1.51	48.93±6.53	53.27±3.25
Ascorbic acid	12.29±1.43	9.12 ±2.36	12.35±1.25
Gallic acid	8.35±1.36	7.68±1.25	8.24±1.32

Means ± SD, (n = 3), whereas $^{\alpha}$ = $p<0.01$,Þ= $p<0.05$.

Because of the positive benefits on human health, herbal supplements and medications are used by people all over the globe (Ahmad et al., 2014). For a long time, several pine species' bark, needles, pollen, and other components were used as high-quality raw materials for making items (Briskin, 2000).

Although they are readily available in many regions of the globe, conifer shoots are almost never utilized as a culinary element. Juices and dairy products with Pinus extracts added have a higher antioxidant potential, suggesting that it might be used for more than just flavoring and scent. By preventing the development of microbes and the oxidation of lipids, pine extract served as an additive that prolonged the shelf life of bread and meat.

The high fat content of pinus seeds gives them a greater energy value. With the exception of vitamin C, which is more abundant in pine needles, the seeds often contain the greatest concentration of the examined components. Pinus seeds include magnesium, phosphorus, and zinc, in particular (Burt, 2004).

According to Miranda et al. (2012), macronutrients have an impact on biochemical reactions, physiological responses, and the amount of yield. The function of plants is dependent on a wide variety of life activities, many of which are influenced by macronutrients. Since components behave in such a complicated manner, it is very difficult to precisely identify their roles. Conversely, micronutrients have a more narrow function in relation to growth and some other well defined plant life activities.Deficiencies in nutrients may disrupt a plant's regular growth and development in a number of ways.

Pine essential oils are said to include around fifty different components. Their concentrations vary in Tables 5-7 based on factors such as plant type, crop, distillation process, and plant portion. These phytochemicals have a wide range of uses and applications due to the wide range of biological activities they display, according to studies. In addition to their antiviral and antibacterial properties, they have a wide range of flavouring and aroma applications. The medicinal and nutritional benefits of α- and β-pinene are not the only ones. Polymer synthesis makes advantage of these adaptable chemicals (Desouza et al., 2007).

To put it simply, polyphenols are secondary metabolites that are absolutely necessary for plant growth and development. Insects and other environmental stresses are also mitigated by them. Plant polyphenols have sensory-related roles, including color, bitterness, and sourness. Flavonoids and simple phenols are the most common types of naturally occurring phenolic compounds. The most frequent class of these chemicals also includes flavonoids. Phenolic acids are in great demand across various sectors due to their role as building blocks for other vital bioactive chemicals that are often required in the food, cosmetic, and medicinal industries, among others. Supplements containing phenolic acids are also sold in stores. Polyphenols may be extracted from pine needles, seeds, bark, and cones using a variety of solvents. Most studies have focused on the pine bark (Masango, 2005; Nergiz and Donmez, 2004).

One of the main ways in which polyphenols work is by lowering levels of reactive oxygen species and acting as antioxidants (Fisher, 1991). Plant polyphenols also have anti-inflammatory, anti-allergic, anti-atherosclerotic, anticoagulant, and antimutagenic actions, which are good for your health [16]. The average person consumes between 0.1 and 1.0 g of polyphenols everyday. The most common foods that contain polyphenols include fruits, vegetables, spices, herbs, coffee, tea, and wine. The area of the peaks of the compounds was determined by comparing the GC-MS chromatograms, and the necessary information was retrieved from the NIST library database [Tables 6-8]. Essential oils of pinus needles and resins are identified by the chromatogram's conspicuous peaks as including a few crucial fatty acids. Because resins and needles have distinct physical structures, it has been suggested that the varied chemical components of essential oils might be attributed to the differing contents of the two (Miranda et al., 2012). While oil's chemistry is complicated and varied, its composition has a direct impact on the efficacy of biological activities, which have displaces differences in its contents depending on the growing location. Chemical components of plants cultivated in various regions of the globe may vary due to seasonal fluctuations (Burt, 2004).

Plants have an important role in preventing many degenerative illnesses due to chemical components that have antioxidant action. Herbs and other dietary supplements for humans have more free radical-fighting chemicals (Farjon, 2018). The antioxidant capacity of the plant extracts was evaluated using three different methods: DPPH scavenging, ABTS scavenging, and H_2O_2 scavenging bioassays. The plants' methanolic extracts effectively scavenged free radicals. By comparing the outcomes of the DPPH and ABTS bioassays. It was anticipated that the DPPH bioassay would reveal much higher antioxidant capacity for the three plant extracts than the ABTS bioassay. According to Tables 9 and 10, P. Roxburghii had a greater antioxidant capability than the resin extracts that were studied.

According to several writers in the literature, plant extracts have free radical scavenging capability and might be a substantial antioxidant agent because of their outstanding reactive oxygen species (ROS) scavenging activity. This was shown by DPPH and other scavenging bioassays. Nitrous acid (HNO_2), hydrogen peroxide (H_2O_2), and free radicals like nitric oxide and superoxide ions are all part of reactive nitrogen species (RNS), whereas reactive oxygen species (ROS) are a kind of activated oxygen. More than a hundred illnesses, including cancer, heart disease, stroke, diabetes, and malaria, have been linked to reactive oxygen species (ROS) and reactive nitrogen species (RNS). The presence of different active secondary metabolites may explain the antioxidant and antibacterial properties shown in pinus needles leaves (Feng et al., 2011).

Similarly, has shown a substantial degree of antioxidant potential in the present study. Research in other regions of the globe, however, has shown that pinus needles leaves have anti-inflammatory and antioxidant properties.

Plant extracts exhibited dose-dependent scavenging of H_2O_2. The oxidation of linoleic acid emulsion with extract revealed that pinus needles had a significant total radical scavenging activity, including superoxide and hydroxyl radicals. The effectiveness of this scavenging activity was dose dependent and it suppressed the production of H_2O_2 at a dose concentration of 300 µg/mL, a characteristic of chain-breaking antioxidants. Some phenolic components that have antioxidant effects include flavonoids, phenolic acids, and phenolic diterpenes. The phenolic component of extracts may be used for scavenging since it can donate an electron to H_2O_2, making it neutralize water. Even while H_2O_2's reactivity is low, it may sometimes be harmful by elevating hydroxyl radicals inside cells. According to Newman et al. (2000), food products must expel H_2O_2.

There has been research on the potential antibacterial properties of pinus needles leaves for quite some time. Many researchers have shown that the respiratory tract benefits from the aroma of pinus needles. Research on the anti-mycobacterium effects of pinus needles led to the recommendation of these tools. The need to find new antimicrobial compounds, whether they be synthetic or natural, is growing due to the rising number of infectious diseases, the serious side effects caused by taking too many antibiotics, and the rise of antibiotic resistance (Alanis et al., 2003).

Traditional medicine practitioners have used plant resin as a remedy for illness for countless years. Prior to the development of contemporary antibiotics, it was also used by the pharmaceutical sector. There may be a connection between the antibacterial properties of Pinus tree extracts, oils, and resins and a number of organic plants.

The Pinus chemical compounds have been shown to have biological effects, which means they might be used to make medications that are both biocompatible and kind to the environment (Edelman et al., 2016).

CONCLUSION

Various parts of *pinus* tree contains different bioactive compounds. Green and dried Needles leaves , resins and cones are important sources of food materials as well as material required domestically. Although pine bark extracts are readily accessible, no one extraction procedure will work for all phenols. To choose the best extraction method, it is necessary to conduct a personalized evaluation that takes into account the desired outcome of the treatment. A high concentration of polyphenols is present in all pine extracts, regardless of the solvent, technique, pine species, or plant portion used. There are encouraging instances of pine components being used in cuisine. Food items may acquire desired attributes, have their shelf life extended, and have useful features via intermediates such as pine tree extracts and syrups. The pharma, cosmetic, and food sectors might all benefit from using pine oils and extracts in their formulations. To address the energy needs of the future, pinus resins may be refined into various fuels such as biodiesel.

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