BROWN GOLD: A LOW COST AND SUSTAINABLE ALTERNATIVE TO DEVELOP PAPER PULP

Submitted in fulfillment of the requirement for the degree of

Bachelor of Technology

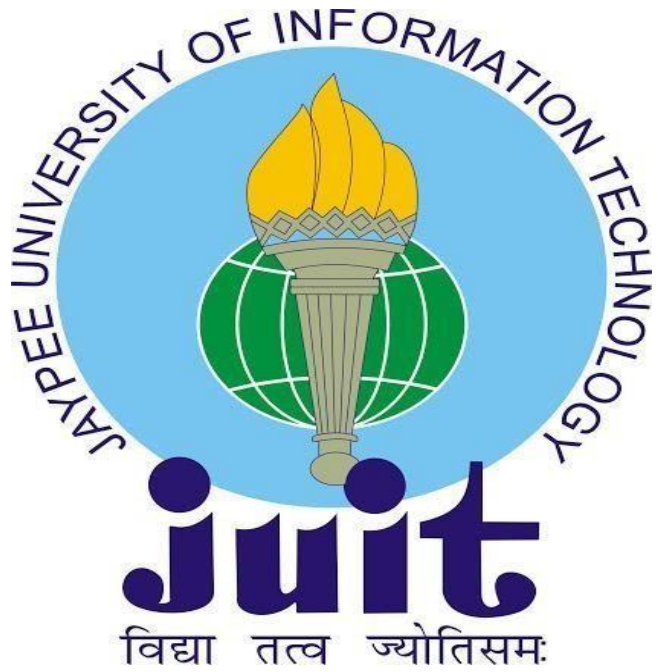
In

BIOTECHNOLOGY

RAMNEEK KAUR KHURANA (181821)

UNDER THE SUPERVISION OF

DR. HEMANT SOOD



DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS

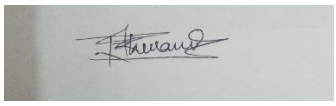
JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

SOLAN (H.P)

TABLE OF CONTENT

DECLARATION

I hereby declare that the project work entitled "Brown gold: a low cost and sustainable alternative to develop paper pulp" submitted to the Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology Solan (H.P), a research work that is carried out is unique and has not been done in this way before. The work is carried out under the guidance of Dr. Hemant Sood.



Ramneek Kaur Khurana

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SUPERVISOR'S CERTIFICATE

This is to certify that the content of this project entitled "Brown gold: a low cost and sustainable alternative to develop paper pulp" by Ms. Ramneek Kaur Khurana is her research project work carried out under my supervision, of the Biotechnology and Bioinformatics branch at Jaypee University of Information Technology, Solan, HP in the academic year 2021-22 for the fulfillment of the requirements of B. Tech Major Project. On the basis of the declaration made by her, I recommend this project report for evaluation.

Signature of the Supervisor

Name: Dr. Hemant Sood

Date:

ACKNOWLEDGEMENT

Foremost, I would like to express our sincere gratitude to the Department of Biotechnology and Bioinformatics of my university for its continued support and encouragement, and also for providing great learning opportunities throughout.

The completion of this project could not have been accomplished without the support of my mentor Dr. Hemant Sood. I cannot thank her enough for her patience, motivation, and immense knowledge. Her guidance has helped me in doing the research work for this project and I couldn't have imagined a better advisor.

Last, but not least, I would like to thank my parents for their support, care, prayers, and sacrifice and for showing concern even during the hectic times.

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Today world is searching for renewable sources of energy. It's searching for alternatives to plastic, cutting trees, cutting down pollution, eliminating agriculture waste, combating climate change, etc. One of the best alternatives to these problems can be pine needles and agricultural wastes. Pine needles are considered as brown gold collected from Pinus longifolia trees composed of 43% lignin,52% holocellulose, and 5.7% extractive contents. Whereas agricultural residues generated from the cultivation of crops include crop leaves, stalks, seeds, fruits, etc. This study is aimed at providing a low-cost and sustainable alternative to wood pulp primarily for developing paper sheets for making articles like packing bags, paper table mats, etc. The experiment was conducted initially by collecting dried pine needles and converting them into a pulp and further different concentrations of waste paper (10% to 40%) and agricultural residues (10 to 40%) were experimented with different concentrations (10% to 50%) of grinded pine needles added into the pulp for making durable paper sheet. The best result in order to develop durable paper sheets has been achieved in the paper having a concentration of 50% pine needles with 20% waste paper and 30% agriculture residues. Moreover, in order to develop antimicrobial characteristics of handmade paper, extract of Picrorhiza kurrao was coated with different concentrations along with different solvents against Bacillus Subtilis and Staphylococcus aureus to measure the zone of inhibition. The results showed non-significant activity. The present study is reported for the very first time where pine needles were tested with waste paper and designed agriculture waste for the development of handmade paper and generating its scope for commercialization. This study also provides a way to eliminate the grave danger of forest fires, increase the local economy, elimination of agricultural waste, recycling of waste paper, and a lot of other environmental problems by utilizing these dried pine needles as a potential alternative.

CHAPTER 1

INTRODUCTION

Pine trees are evergreen, coniferous trees. The bark is thick and scaly but may vary according to species. These trees grow up to 50-150 ft tall. These trees are used for timber and wood fuel. Pine needles have many uses, whether they are cut from the tree, freshly fallen, or dried. Pine needles are commonly used in gardening and landscaping, for ornamental purposes. It is used by various people as a pine needle handicraft *(like a basket, tray pots, etc)*. [2] These pine needles are also used for cooking and baking purposes. Moreover, pine trees have inner to outer wood bark moist cambium layer which is edible and rich in vitamin A and C. Pine needles have cellulose(>50%) and lignin as main components. [1]

The northern Indian state of Himachal Pradesh situated in the western Himalayas is home to scenic mountains and has picturesque towns and villages. It is full of lush green forests accounting for two-thirds of the total geographical area legally classified as forest. As per a forest survey of India 28 % i.e., around 15434 square Kilometers is covered with forest. There are Chir pine, deodar, oak, and kail tree forest found in Himachal. [3]

However, this beauty has a price. During summers the forest areas especially Chir pine forests are vulnerable to wildfires causing a huge loss to the local economy, and forest biodiversity causing pollution. Three species of Pinus plants are abundantly found in Himachal Pradesh i.e., *Pinus roxburghii, Pinus wallichiiana, and Pinus gerardiana*. There are frequent forest fires in the regions of Dharamshala, Kullu, Shimla, and other parts of the state. There were 845 incidents of forest fires reported alone in April 2021 [4]. Pine needles shed 1/3 of their leaves in summers and are replaced by new ones and these fallen down pine needles dry and become inflammable causing huge loss to flora and fauna. Data from the Himachal Pradesh Forest department states that an area of 1,25,885 ha is estimated to be covered by pine forest and 1.2 tones of pine needles are shed per hectare annually.

According to the forest department, most fires are caused by human negligence and most fires start in and around habitable zones. Dried pine needles cause trouble in collecting fodder and so the village people often collect the dried pine residue and set it on fire, in no time these fires turn into huge forest wildfires causing hazardous smoke making it difficult to breathe and live in the area.

Pine trees also produce certain biochemical which does not allow other variety of vegetation to grow. Many environmentalists have suggested replacing Chir pine trees with broad-leafed trees yet there is no planned solution till now.

This research focuses on contributing to the solution of this grave danger of forest fires, along withconcentrating on the elimination of agricultural waste and recycling paper making this research is in terms with the United Nations SDGs 11 (sustainable communities and cities), 13 (climate action). Furthermore, its focus is on Atama Nirbhar Bharat and self-reliant Himachal Pradesh.

Agriculture waste used in the research was optimized by using a different combination of kitchen waste which includes pulp, peels of fruits and vegetables along with plant residues collected from the herbal garden of the department. These residues have a good amount of cellulose and lignin content required for paper production. The optimized waste paper used i.e., used tissues, old used paper, etc. Waste papers consist of approximately 40-80% of cellulose, 5-15% of hemicellulose, and amounts of lignin and proteins. All this contributes to the formation of durable paper without using any chemicals to form paperbags and other items.

In the present study the research carried out is an innovative approach to tackle the problem of forest fires caused by these dried pine needles along with the utilization of optimized waste paper and elimination of agriculture waste.

This research was even extended to make these paper food packaging ready by coating it with medicinal plant extract of P. kurrao which was invitro grown in the Biotechnology department of the university. The paper was then checked for antimicrobial activity by the disc diffusion method against B. subtilis and S. aureus. These bacteria are high in salt tolerance and can grow on meat and dairy products also these are heat resistant and cannot be killed by cooking.

However, the results were non-significant. The plant extract showed very little activity against these microbes.

CHAPTER 2

REVIEW OF LITERATURE

To carry out this research work various research papers were reviewed in the context of pulp and paper formation from various sources like rice straw, pine needles, wood, etc. We reviewed various methods and processes via citing different research papers and reports.

2.1 Pine Needles as potential energy feedstock [5]

L.D Kala* et al of the mechanical engineering department at Indian Institute of Technology Delhi,Hauz Khas, New Delhi carried out research and used pine needles as an alternative to fuelwood to generate electricity to meet the electricity demand of the area. The research was published in 2017 and published at the world renewable energy congress.

Further, they comprehensively estimated the pine needles yield and also compared the calorific value of fuelwood, rice husk pine needles, etc., and their availability.

This study was brought to focus energy situation in the state where demand exceeds supply. Almost 17 percent of the population did not have excess electricity. The year 2014-15 recorded the peak electricity demand at 1930 MW and the peak energy shortage between 8 and 46 percent. The disadvantage of pine needles to catch fire was used for gasification of pine needles to produce fuel gas for the production of electricity. The study estimated that the annual gross (overall produced) pine needle yield was 1.9 million tons while the annual net (used for production) pine needle yield at 1.33 million tons. It was estimated that electrical energy generation from pine needles using thermochemical conversion (Thermochemical conversion of biomass can be performed via three primary pathways, i.e., combustion, gasification, and pyrolysis (thermal decomposition of biomass occurring in the absence of oxygen), i.e., use of heat), an installed capacity of 789 MW can be supported with pine needles feedstock for supplying electricity in rural areas for five hours a day.

The annual primary energy potential of pine needles in the state has been estimated at 25.9 petajoules (10^15). The annual net energy is estimated at 17.1 PJ for thermal applications. Alternatively, an estimated amount of 1438.9 million kWh of electrical energy can be obtained. This is a significant amount as the commercial sector in Uttarakhand consumed 1411 million kWh in the year 2013-14.

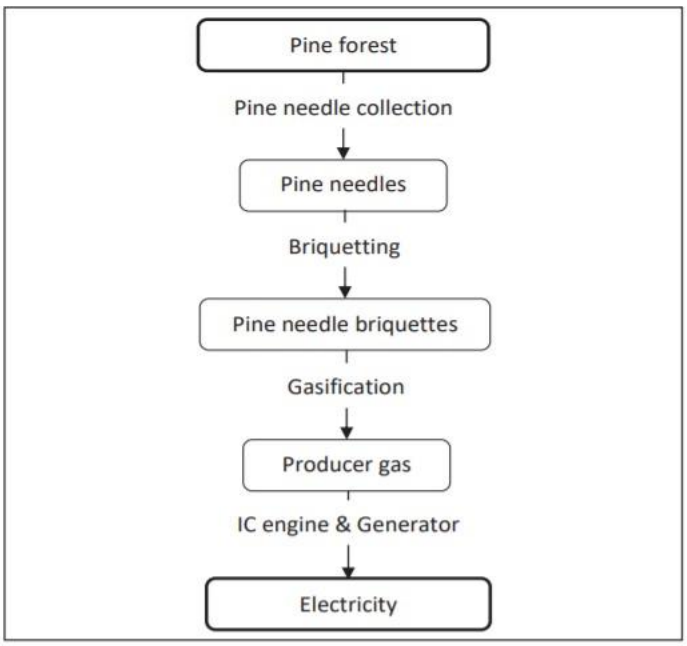


Figure-1: Flow Chart depicting process of power generation from pine needles

Table-1: Comparision of pine needle as gasification feedstock

Name feedstock	**Calorific value (MJ/kg)**	**Bulk density (kg/m^3)**	**Source**
Fuelwood	7.2-14.4	188-277	[10]
Pine needle	19.44	94.33	[11]
Bagasse	14.6	935.6	[12]
Rice husk	13.24-16.2	86-114	[13]

Table 2.1.2: Pine needles yield per kg/ha/yr

S. No.	Source reference	Needle yield (kg/ha/yr)	Pine tree per ha	Needle yield per tree (kg/yr)	Remarks
1	[21]	5994	575	9.2	-
2	[22]	5371	278	19	-
3	[22]	5700	1630	3.5	Tree density high.
4	[22]	6800	657	10.4	-
5	[22]	7400	820	9	-
6	[22]	7600	540	14.1	-
7	[23]	8900	700	12.7	-
Average value of pine needle per tree = 12.4 kg					

Ecofriendly use of hazardous pine needles for social benefits [6]

Pine needles are the major cause of forest fire, loss of forest biodiversity, and local economy. Observing this IIT mandi introduced a pilot project of palletization of pine needles that are to be used as fuel, thereby countering the harmful effect of highly inflammable, non-biodegradable pine needles.

They set up a briquetting unit with a capacity of 150kg/hour with a connected load of 12 HP. This was a 2-year project that started on 25 October 2018.

Advantages of briquettes

- Low in sulfur
- 3 Kg of pellets will emit equal heat as 10 Kg of wood emits

Fig: 2.1.2 Pelletization of pine needles

2.2 Pine needles- an evaluation of pulp and paper making potential [1]

Priti S. Lal et all carried out research to evaluate the pulp and papermaking potential of pine needles.

Pine needles collected from the hilly regions had 43% lignin, 52% holocellulose, and 5.8% extractive content. They carried out Kraft cooking of pine needle using 24% active alkali resulted in the unbleached pulp of kappa number *(The Kappa number is **a measurement of standard potassium permanganate solution that the pulp will consume**. if the kappa number value is higher then, **the required bleaching chemicals are also higher**. On the other hand, low kappa pulps are easier to bleach.)* 28 and unbleached pulp yield of 24%.

They collected pine needles and evaluated them based on various factors like ash content, NaOH solubility, lignin, holocellulose content, etc by various methods.

Further, the pine needles were subjected to chemical digestion at 168 ℃ Then bleaching of pulp was done based on various parameters like consistency, reaction time, reaction temp., and pH maintained.

After the paper formation, the physical properties of the paper were evaluated. Tensile strength, baseweight, tear index, folding index, opacity, etc were checked.

Table 2.2.1: Parameters of the evaluation

Physical Strength Properties of both Unbleached and Bleached Pulp

Parameters	PFI (rev)	Freeness ml,CSF	Apparent Density g/ cm3	Burst Index $KPam^2/g$	Tensile Index Nm/g	Fold Kohler Molin(log)	Porosity Bendtsen (ml/min.)	Opacity (%)
Unbleached pulp	nil	250	0.85	3.50	59.5	2.24	78.5	-
Bleached pulp	nil	205	0.93	3.20	57.5	2.06	68.5	79.2

Pine needles have more than 50% holocellulose, and hence can be used for the production of pulp and paper. Though it contains higher lignin which results in high bleach chemical demand. Instead of bleached variety, it can be utilized for the production of semi bleached grade pulp, unbleached kraft paper, filler, or paper/board.

2.3Antimicrobial Studies [8]

P.Vinoth Kumar et all carried out the in-vitro antibacterial activity of P kurrao rhizome extract using the agar well diffusion method. They used various solvents like acetone, methanol, ethanol, etc for the rhizome extract of P kurrao and it was tested against strains belonging to genera of gram-positive and negative bacteria. The research found that the ethanol rhizome extract showed high activity against S. aureus and hexane and acetone showed intermediate activity against them. However, the aqueous extract showed no activity against any bacterial strains.

2.4 Phytochemical Screening [9]

In another experiment carried out by D Rathee et al, phytochemical screening of the rhizomes of Picrorrhiza kurroa Benth was done which showed the presence of bioactive components, which have been linked to antimicrobial properties. They performed various chemical tests and TLC studies which revealed the presence of glycosides, sterols, and phenolic compounds. The major chemical constituents found in this plant were iridoid glycosides and cucurbitacins (triterpenoids) present in the methanolic extract.

These compounds when suspended in methanol showed activity against certain bacterial and fungal strains like Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Candida albicans, Aspergillus niger, respectively. The experiment also confirmed the effectiveness of the crude extract that are used in traditional medicine to treat skin, urinary tract, diarrheal infections, etc. Further, it was found that the aqueous extract was less effective against the microbial strains and had no activity against fungal strains.

2.5 P. kurrao extract from seeds [10]

Seeds of P . kurrao also has the potential for antimicrobial activity. The research was carried out by Jannathul Firdous et all in Malaysia and the activity of compounds was evaluated using TLC and FTIR. The seeds were collected and the extract was made out of them and was first defatted with petroleum ether followed by the extraction with ethanol and an alcoholic extract was made. The extract was checked against various bacterial strains like P aurenginosa and E. Coli. And S aureus etc. The results were significant and various zones of inhibition was obtained. The maximum is 5.0mm.

CHAPTER 3

RATIONALE OF THE STUDY

1. Pine forests are abundantly found in the Himalayan belt starting from Jammu and Kashmir to North East. Hence, there are several cases of forest fires reported that cause loss of biomass, and life and even contribute to pollution.
2. There is also a problem of plastic pollution as well and the world is searching for renewable resources, it's searching for means to curb pollution, eliminate agricultural waste, combat climate change, etc.
3. To provide solutions to real-life problems and form biodegradable products out of pine needles along with using agricultural waste and also recycle waste paper.

OBJECTIVE

1. To optimize conditions to form paper pulp using pine needles and agriculture waste.
2. To optimize the basic condition for making handmade paper having antimicrobial activity.

CHAPTER 4

MATERIALS AND METHODOLOGY

4.1 PREPARATION OF PULP FROM PINE NEEDLES [7]

4.1.1 Collection of pine needles: The pine needles were collected from the chir pine forest near the university. (as in fig 4.1.7 a)

4.1.2 Washing of pine needles:

The collected pine needles were washed to remove the dirt and dust with normal water and then with distilled water. After that, the needles were chopped and cut into small pieces using scissors.

4.1.3 Boiling & washing

The chopped needles were then subjected to boiling in a big container at 100℃ for 2-3 hours to make them soft and tender and break the fibers, and lignin, and make them ready for grinding.

(The result is in 4.1.7 b)

4.1.4 Grinding

The boiled needles were washed and grinned in the mixer grinder issued from the department and were converted into a fine pulp. (The following result is in 4.1.7 c)

4.1.5 Spreading

The pulp was then suspended in the bucket half-filled with distilled water and a fine liquid was formed.

4.1.6 Molding

For molding and shaping of paper, a round sieve that was issued from the department lab was ducked in the bucket and was slowly lifted making sure the mixture was passed through it. The excess water was drained out and the remaining was pulp which was pressed using a muslin cloth and removed slowly from the sieve. It was then kept for drying and the paper was obtained. (The result is in fig 4.1.7 e and f)

This methodology was carried out with pine needles along with agriculture waste and optimized waste paper.

a) COLLECTION b) BOILING c) GRINDING

f) PAPER FORMED e) MOLDING d) PULP FORMED

Fig 4.1.7 Schematic representation of the methodology

4.2 PREPARATION OF PULP FROM PINE NEEDLES AND WASTE PAPER

While carrying out this phase of the experiment varied concentrations of pine needles were taken along with waste paper to derive a durable paper. (The details are mentioned in table 4.2.1)

Table 4.2.1: different concentrations of pine needles and waste paper for developing paper pulp

S.NO.	PINE NEEDLES CONC. (g)	OPTIMIZED WASTE PAPER CONC. (g)
1	80	20
2	60	40
3	50	50

4.3PAPER PRODUCED FROM PINE NEEDLES, WASTE PAPER, AND DESIGNED AGRICULTURAL WASTE

While following the same procedure pine needles were experimented with varying concentrations of the pine needles, waste paper, and agriculture waste which includes, pulp and peels of fruits and vegetables, field residues like stocks, and left out plants from the herbal garden of the department. (The result of this is in the table 4.3.1)

Table 4.3.1: Different concentrations of pine needles, waste paper, and agricultural waste

S.NO.	PINE NEEDLES CONC. (g)	OPTIMIZED WASTE PAPER CONC. (g)	AGRICULTURE WASTE CONC. (g)
1	60	20	20
2	50	20	30
3	40	30	30

4.4 ANTIMICROBIAL FOOD PACKAGING

Packaging is the technology by which enclosing and protecting products for distribution, storage, and sale are done. It ensures food travels safely to longer distances and even increases its shelf life. The primary objective is to protect food against water, ultraviolet rays, pathogens, etc. Interest in the production of food packaging from biodegradable materials is constantly increasing across the globe. Consumers demand products that are environmentally friendly, safer, and nontoxic. Today, almost all food is packaged for sale to the consumer. Food packaging is essential for the preservation and protection of food from contamination.

Different approaches have been applied for developing antimicrobial packages, including antimicrobial sachets inside packaging, packaging films, edible coatings incorporating active antimicrobial substances, etc. While all these antimicrobial packaging systems are effective to some extent in preserving quality and ensuring microbial safety of fresh and minimally processed produce. (8)

The project extended to produce antimicrobial food bags out of pine needles, agricultural waste, and waste paper by coating the bag with an extract from the medicinal plant – Picrorhiza kurrao.

4.4.1 PICRORHIZA KURRAO

It's an endangered medicinal plant of the alpine Himalayas. It acts as a hepatoprotective agent, immunomodulatory, and anticancer agent. It has been used for curing various diseases in Ayurveda.

It also has antimicrobial activity which was found effective against Staphylococcus aureus, Bacillus subtilis, etc. for carrying out antimicrobial activity these stains were procured from a departmental microbial repository, and their cultures were maintained by following biosafety levels 1 and 2, and disposal was also carried out by following biosafety protocols.

For the development of antimicrobial paper for food packaging in the present study in vitro grown p. kurrao was used. (9)

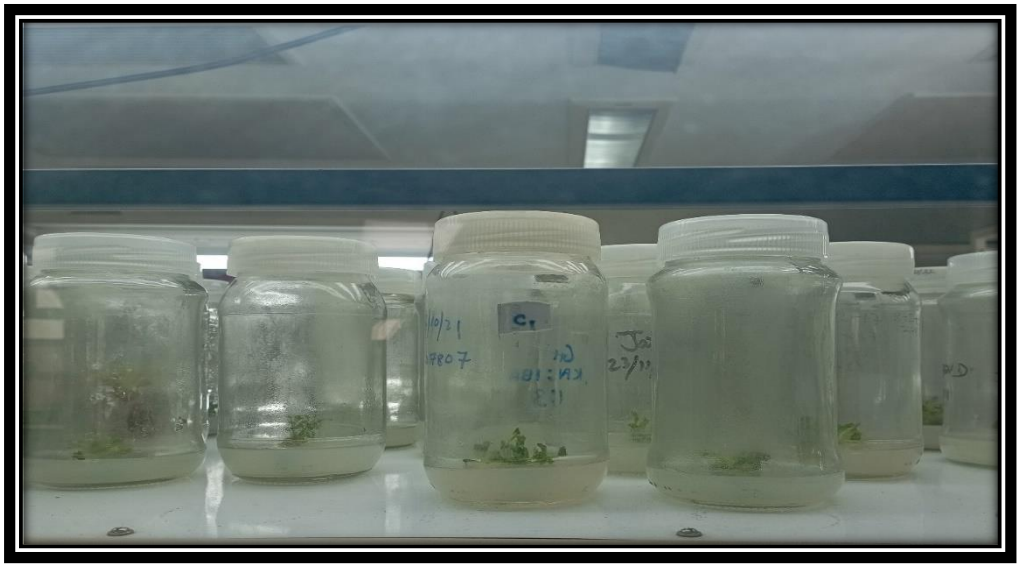


Fig 4.4.1: Invitro shoot multiplication of P. kurrao under optimized conditions

The following steps were followed for the same. The Invitro grown was multiplied with the optimized condition as mentioned by Sood et all in 2009. The micropropagation of shoots was carried out (Fig 4.4.1)

4.5 PREPARATION OF PLANT EXTRACT

STEP 4.5.1. The lab-grown P. kurrao was taken and washed with distilled water to remove the media and kept to get dried for one to two days. (Result is described in fig 4.5.1)

Fig 4.5.1: Invitro grown shoots kept for air drying

STEP 4.5.2. The dried plants were then made into a fine powder using a mortar pestle and were weighed.

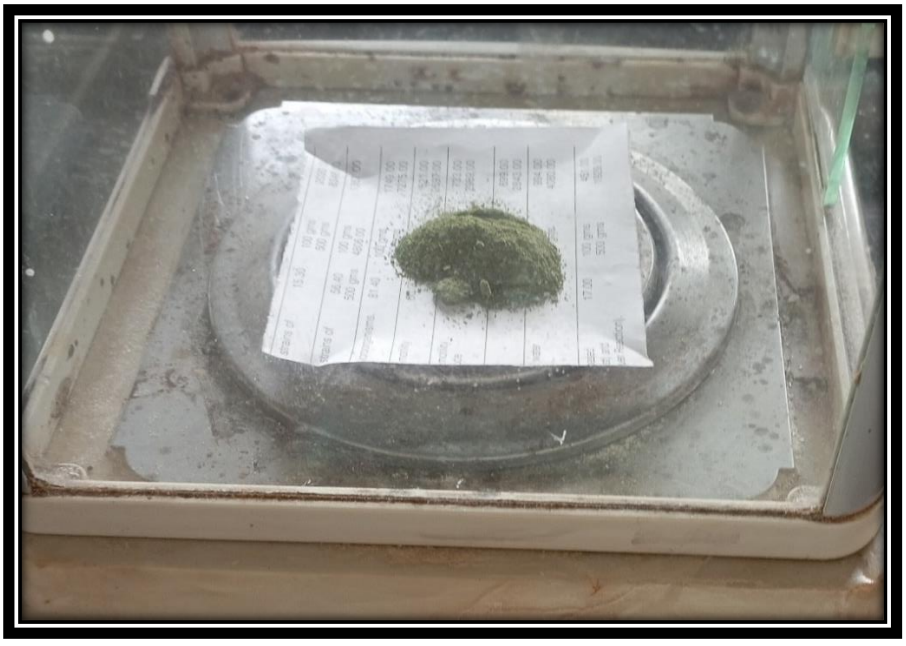


Fig 4.5.2: Powdered plant extract of Picrorhiza

STEP 4.5.3. The powdered extract was then suspended at 80 % 10 ml Methanol so as to dissolve all the phenolic components of the powered plant. The flask was then kept in a shaking incubator overnight as shown in fig 4.5.3.

Fig 4.5.3: The methanolic plant extract of P kurrao

STEP 4.5.4. The methanolic extract was then filtered out and excess methanol was dried off by spreading the filtered-out extract on a Petri plate and kept to dry.

Fig 4.5.4: Filtered and dried P.kurrao extract

STEP 4.5.5. The dried extract was then scraped off and powered was stored in the eppendorf until further use.

4.6 Antibacterial Activity

The agar disk diffusion method was used to determine the antibacterial effect of P. kurrao extract against S. Typhi, S aureus, and B. Subtilis. This method is a qualitative method to check whether the agent can work against pathogens or not. Microbes are potent food spoilage organisms and even cause diseases in humans.

METHODOLOGY (8)

1. PREPARATION OF AGAR PLATES

300ml of Muller Hinton media were prepared. 6.3 g of MHB was weighed and added to a flask and mixed with 100 ml distilled water. Then 4.5 g of agar was weighed and added to the same flask and using a measuring cylinder volume was made up to 300 ml and mixed till the MHB and agar dissolves in water. The flask was covered tightly with a cotton plug and covered with paper completely to make it ready for autoclaving.

2. PREPARATION OF NUTRIENT BROTH

100 ml of nutrient broth was prepared by weighing 1.3 g of nutrient broth and mixing it with 100 ml of distilled water. Out of it 10 ml was added to each of 10 test tubes and covered tightly with a cotton plug ad was autoclaved.

3. POURING OF PLATES.

All this was carried out in an antiseptic environment under a Laminar airflow chamber.

4. PREPARATION OF BACTERIAL CULTURE

The autoclaved test tubes with the nutrient broth were taken and inoculated with 20 microliters of bacterial culture of B subtilis, S aureus, and S. Typhi. Then the test tubes were kept in a shaking incubator overnight for the growth of the culture. The growth of the culture was checked by checking the turbidity of incubated test tubes with the control test tube which only had nutrient broth.

5. Preparation of paper discs

The paper which was made out of pine needles, agricultural waste, and waste paper was punched out into small discs and was stored in an Eppendorf which was then autoclaved.

ANTIBACTERIAL ACTIVITY BY AGAR WELL DIFFUSION METHOD

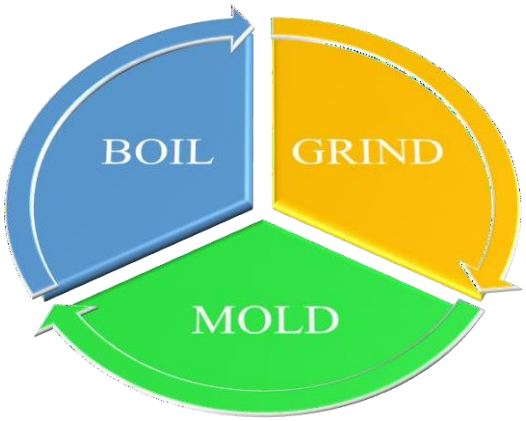
The Agar well diffusion method is a well-known method that is used frequently to determine

the antibacterial effect of plant extracts. Using this method, prepared sterile agar plates were seeded with 25 microliters of standardized bacterial inoculum on agar surfaces by an L-shaped spreader, and a sterile tip was taken to create 2 uniform wells one for test solution and another one for control. (11)

The autoclaved paper discs were added to the wells. Then 100 microliters of plant extract were added on one side and the control solution of methanol was added on the other side. All the steps were performed in a Laminar air flow chamber to avoid contamination. The plates were covered by parafilm and were incubated for 24 hours at 37 degrees.

CHAPTER 5

RESULTS AND DISCUSSION



5.1 Paper developed by using pine needles

The experiment was conducted for developing paper pulp by using only pine needles. As we used different concentrations of pine needles for developing pulp. The present study reported that 100g of pine needles can produce 12 sheets of paper weighing 8g each. The paper produced from only pine needles was less durable and tears easily.

Fig 5.1a: Paper produced from pine needles and Fig5.1 b: shows breaks in paper

5.2 Paper produced from pine needles and optimized waste paper:

In this, the different concentrations of pine needles along with the varied concentration of waste paper was used. It was found that the durability of paper increased as the concentration of waste paper increased. It was observed that 50g pine needles along with 50g optimized waste paper provided durable paper. (The different concentrations are in table 4.2.1)

Fig 5.2 a: Paper with 80g pine needles and 20 g waste paper

Fig 5.2 b: Paper having 60g pine needles and 40 g waste paper

Fig 5.3 c & d: Paper with 50 g pine needles and 50 g waste paper formed into bag

5.3 Paper produced pine needles, optimized waste paper, and agriculture waste:

To make the paper different concentrations of pine needles, waste paper, and designed agriculture waste was used. The agriculture waste included peels and pulp of vegetables, fruits, etc., and field residues like stalks, dried leaves, and shoots from the herbal garden of the department. The most durable paper was formed with a concentration of 40g pine needles, 30g

Optimized waste paper, and 30g agriculture waste. (The concentrations are described in table 4.3.1). The paper thus formed was better than the paper formed from just pine needles and waste paper (Fig 5.2 a &b) much better than paper formed with only pine needles (Fig 5.1 a and b)

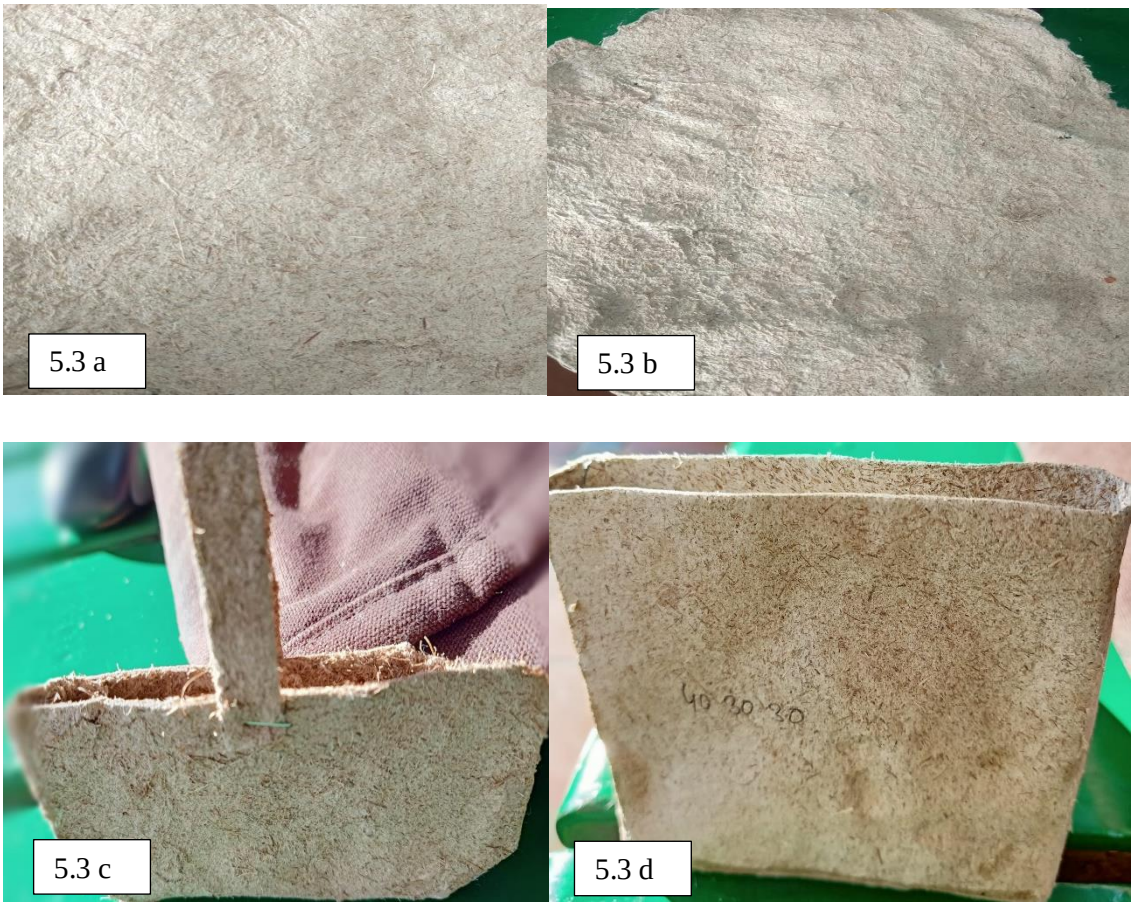


Fig 5.3 a: Paper with 60 g pine needles, 20 g waste paper, and 20 g agriculture waste.
Fig 5.3 b: Paper with 50 g pine needles, 20 g waste paper, and 30 g agriculture waste.
Fig 5.3 c & d: Paper with 40g pine needles, 30 g waste paper, and 30 g agriculture waste formed into paper bags.

5.4 Antibacterial activity

The method of agar well diffusion was performed to see the antimicrobial activity of P. kurrao. The expected result of getting a zone of inhibition was not observed. The experiment was repeated many times with different concentrations of plant extract and using different solvents like ethanol, acetone, isopropanol, and methanol. A less significant result was observed when methanol was used as a solvent. Some activity was observed but it was not remarkable.

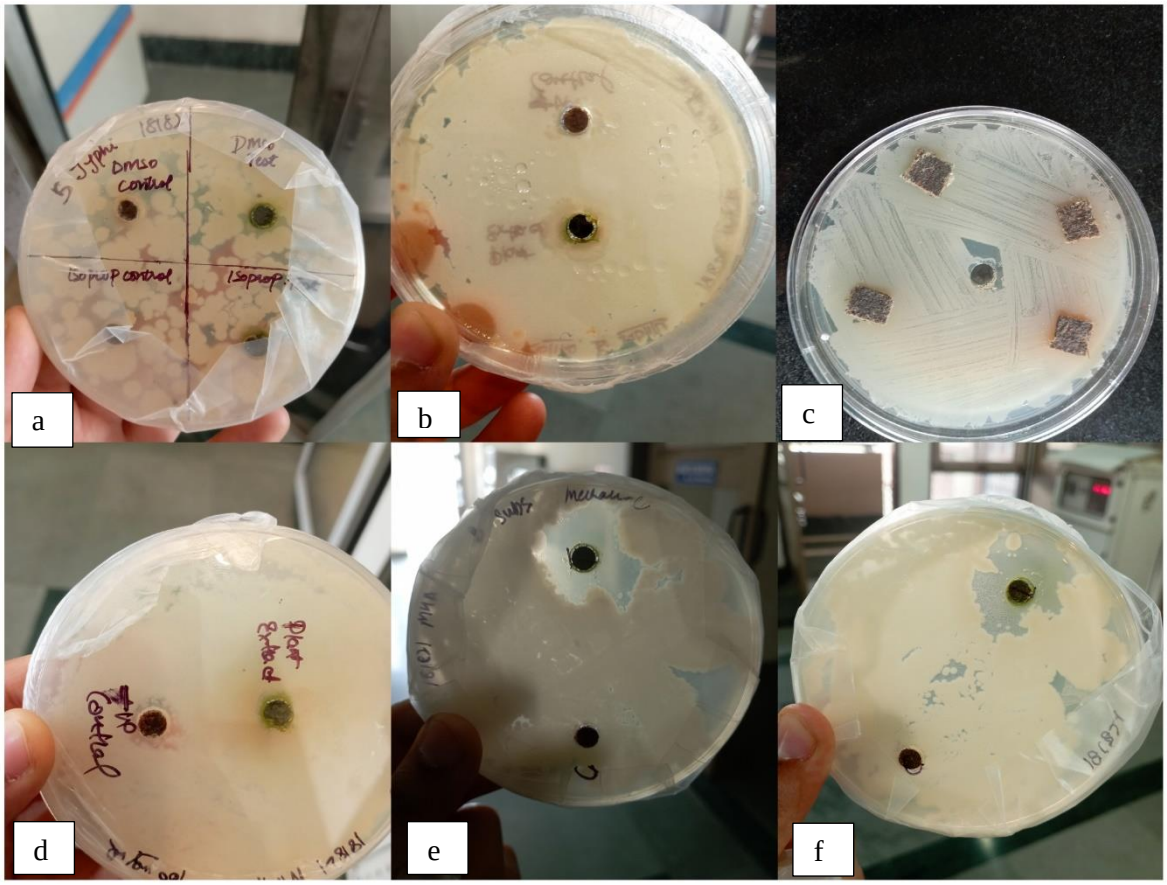


Fig 5.4: Non-significant results of antimicrobial activity

Figure 5.4 (a), provides the result of plant extract made in DMSO, isopropanol reporting not result but contamination. In the (b) the concentration of plant extracts was 100m/ml showing no zone of inhibition. In the next Petri plate, the solvent used solvent for plant extract and control was acetone with nonremarkable results. Further in the next two figures, a distorted zone of inhibition was observed. The solvent used was methanol.

All the results were performed from two bacterial strains, Bacillus Subtilis, and S. aureus which was approved and permitted for use by my project mentor and were procured from the department.

DISCUSSION

Paper is the most important thing for humans and to produce 1 ton of paper approximately 24 trees are cut down. Today there is a need for an hour to look for renewable resources of energy. There is increased deforestation, a lot of agricultural waste, plastic pollution, etc. and all of this is contributing to climate change. There is one more problem prevalent in the Himalayan region that is dried pine needles that are shed in summers and contribute to alot of forest fires. According to the Himachal Forest department in the year, 2019-2020 2500 forestfires were reported causing a loss of forest biodiversity, loss of local economy, and air pollution. These problems can be tackled by an innovative approach and focus on the sustainability of the environment. The above research can contribute towards curing a lot of environmental problems, like utilization of waste paper, elimination of agriculture waste, reduction of the use of polythene bags, etc. in the present study the development of paper pulp was carried out using pine needles which are lying on the vary same reports as reported by Priti S. Lal et all where they used pine needles as the potential source. It was found that unevenness while molding results in hard and worthless paper. Also, the grinding needs to be done properly. Similarly, antimicrobial activity was studied by P. Vinoth Kumar et al. but in this, the plant extract reported less activity against bacteria which was same in the referred research papers. So, in future experimentation, other combinations might be tested for improving the activity.

Fig 5.5- Schematic representation of work

CHAPTER 6

CONCLUSION AND FUTURE PROSPECTS

With advancing technology and increasing human needs we are exploiting nature in a lot of ways. The resources are depleting and our planet is increasing towards non-sustainable development. Today more than fish we have plastics and the marine biodiversity is harmed, countryside today has changed from peaceful sites to dumping grounds for the abundant amount of non-biodegradable waste. Earth's temperature is rising and there is a rapid advancement in the disbalance of climate that is causing unseasonal rainfall, and cyclones every now and then due to a rise in ocean temperature. Intergovernmental Panel on Climate change reported that in around a decade earth's temperature will rise up to 1.5 ℃. There is a need for an hour to focus on different parameters and solutions for the environmental problems around us. We cannot change and solve the issues of the whole earth in one day or even in a decade it's a slow process and we have to start with the issues around us. This research has focused on the rampant forest fires in and around Himachal Pradesh and tried to solve the grave issue along with a solution to eliminating agricultural waste and recycling waste paper. This research also focuses on increasing the local economy of Himachal, planting a seed for biobased industries in Himachal, women empowerment, and giving a low-cost and sustainable approach/alternative to develop various articles. The products formed are biodegradable, easy to make even with less developed equipment, eco-friendly, and cost-efficient way. In the present study, the use of pine needles with waste paper and a combination of agricultural waste was used for the first time for developing handmade paper. So, this study generates many future prospects like making the product food packaging ready, generation skills in rural unemployed, making people aware of its use and protecting forests from forest fires, and contributing towards solving the problem of climate change.

Furthermore, for the improvement of antimicrobial activity P kurrao along with other plants might be tested for improving its scope for food packaging and other desirable articles.

CHAPTER 7

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CHAPTER 8

PUBLICATIONS

[1] Ramneek Khurana and Hemant Sood presented in Shodh Shikhar 2022 with project title Brown Gold: An alternative and cost-effective solution to pulp and paper production organized by United Nations Development Program with Rabindranath Tagore University Bhopal, pp198 from 25-26 March 2022 (Oral Presentation)

[2] Oral presentation at 4 th International Conference on "Challenges in Chemical and Biochemical Engineering for Sustainable Development (CBSD)" at Annamalai University, India. With paper title, Pine needles: A Cost-effective alternate to produce Pulp and Paper.