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Biotransformation based Pharmacophores from Plant Extract of *Picrorhiza kurroa* Royle Ex Benth

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ABSTRACT

An investigation entitled "Optimization of fermentation parameters to enhance biotransformation based pharmacophores from plant extract of *Picrorhiza kurroa* Royle ex Benth" was conducted in the Division of Microbiology, Sher-e-Kashmir University of Agricultural Sciences to optimize different fermentation parameters to enhance the polyphenols in the plant *Picrorhiza kurroa* Royle ex Benth collected from the Galhar region of District Kishtwar of UT Jammu & Kashmir. Four fermentation parameters were optimized during the experiment i.e. temperature, pH, inoculum load and incubation time with *Saccharomyces cerevisiae* and *Lactobacillus acidophilus.* The experimental results revealed that among *Saccharomyces cerevisiae* and *Lactobacillus acidophilus, Saccharomyces* performed better in all the conditions. *Saccharomyces* showed the maximum increase in phenolics and flavonoids. It showed the max increase at 30^0C (92.10 to 127.04mg GAE/g dw) temperature and at pH of 5.5(92.43 to 128.96mg GAE/g dw) When incubated for 48h. Also, it was observed that increasing incubation time has an increasing effect on total phenolic content and flavonoid content but prolonged fermentation results in the decrease of both. In case of *Saccharomyces,* decrease in phenolics was observed after 48h (136.77 to 100.05mg GAE/g dw) but in case of *Lactobacillus,* decrease was seen after 72h, which could be due to the oxidation of phenols by bacteria. Further LCMS analysis also revealed an increase in the quantity of compounds as there is increase in the peak area. Thus, this study suggests optimum fermentation parameters and microorganism for enhancing bioactive compounds in *Picrorhiza kurroa* Royle ex Benth. The results suggest that *Saccharomyces cerevisiae* is the best microorganism for fermentation of *Picrorhiza kurroa* Royle ex Benth and the optimum conditions for incubation is 30^0C temperature at pH5.5 for 48h; that gives improved nutraceutical values and enhance the bioavailability of these bioactive compounds.

Keywords- Fermentation, Picrohiza kurroa, total Phenolic content, Biotransformation, total flavonoid content.

I. INTRODUCTION

J&K state possess a significant portion of Himalayas (Western Himalayas) with areas of high altitude, cold deserts and immense plant diversity. More than 800 valuable medicinal species found in north western Himalayan region is extensively used by the locals, for curing various diseases of human kind. Different parts of medicinal plants are used in treatment of ailments/ diseases of body. *Picrorhiza kurroa* is also an important medicinal plant in Indian medicine system having various pharmacological properties. It is commonly known as 'kutki', belongs to family Scrophulariaceae. The complete botanical name of the plant is *Picrorhiza kurroa* Royle ex Bentham. It is an endangered medicinal plant species found in the indo-china Himalayan region. *Picrorhiza* is a self propagating plant in nature but it's over harvesting has made it to near extinction. This family has around 200 genera and 3000 species, and all these species are mostly found in northern temperate regions of the world i.e. in regions of china, Pakistan, India, Bhutan and Nepal. It is found in abundance in the alpine Himalayan region, in Nepal. *Picrorhiza kurroa* is a perennial herb having elongated

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rhizome, basal and alternate, oval, coarsely toothed leaves 5-15 cm long. its flowers are mostly white or sometimes pale purple and are on a long stalk, bloom in the month of June through August. Rhizomes of the plant are long, grayish brown with rough surface and are irregularly curved as thick as little fingers (Rasool, *et al.,* 2017).

The rhizome of the plant is considered to be valuable bitter tonic, antiperiodic, cholagogue, stomachic, laxative in small doses and cathartic in large doses and useful in leukoderma, snakebite, and other health issues (Rathee *et al.,* 2012). It grows in wild form in alpine regions on rock crevices and also in organic soils (Masood *et al.,* 2015). It is mostly used in traditional medicinal system for asthma, jaundice, fever, malaria, snake bite, and liver disorders. Its different pharmacological activities include anti-microbial, anti-oxidant, anti-bacterial, anti-mutagenic, cardio-protective, hepato -protective, anti-malarial, anti-diabetic, anti-cancer, anti-inflammatory, anti-ulcer and nephro - protective. Because of its versatile uses the demand of this species is continually on the rise, but due to the destructive harvesting and its habitat demolition due to deforestation its supply is inconsistent and inadequate. Till now many phytochemicals have been extracted from this plant including iridoids, cucurbitacins, phenol glycosides, pikuroside, as the major constituents. In India, the extract of its rhizome are considered as an antibiotic and has been used widely in Ayurvedic and Unani medicine systems (Salma, *et al.,*2017).

In ayurvedic system of medicine fermentation is prescribed as a method for drug preparation as fermented foods become an important part of the diet in many cultures because many health benefits has been associated with fermentation. Due to this reason the fermentation process and the resulting fermented products have gained recent interests of many scientists, adding to this are the microorganisms, who are contributing to the fermentation process have also been associated with many health benefits and so becoming the point of focus (Sanlier *et al.,*2017). It is found that fermentation has the potential to enhance the metabolites and various constituents of plant extracts. It is an important technique in bioconversion of bioactive molecules of plant extracts by microorganisms. For instance cell wall degrading enzymes of lactic acid producing bacteria contribute to the maceration of the plant matrix, therefore enhancing the bioavailability of the plant secondary metabolites (Touret *et al.,* 2018). It has been reported that microbes produce an array of primary and secondary metabolites, but they do so in very low quantity, but using fermentation techniques high yields can be obtained (Singh *et al.* ,2017).

Fermented plant extract has many benefits to human, not only to health but also to industry and environment. Nowadays more and more fermented plant extracts are produced on industrial level. With the improvement of living standards, people pay more and more attention to health and longevity. The development prospects of fermented plant extract products have great potential (Feng *et al.,* 2017).

Advancement in medicine has indicated that fermentation can be an important tool for improving potential of active ingredients in drugs of plant origin. Also this technique is used to enhance the functional features of medicinal plants or to diminish their side effects. Traditionally plant extracts are fermented by natural micro flora and sometimes by known strains. Therefore, fermentation can be exploited for increasing health benefits from drugs of plant origin as well as that from food. Several parameters during fermentation affect the type and quantity of targeted bioactive molecules. The conditions include the pre-treatment of the substrate, the selection of the microorganisms to ferment besides the common conditions such as pH and temperature (Prachyakij *et al.,* 2008) and these conditions must be optimized before the fermentation is carried out on large scale metabolite production and is also challenging. By optimizing the above said parameters, maximum product concentration can be achieved (Wang *et al.,* 2011).

Microorganism selection is also an important process in fermentation of plant extract. The microorganism can be a known culture from laboratory and company, or isolated from nature, while traditional fermented plant extracts are usually fermented spontaneously (Feng *et al.,* 2017). Increase in productivity reduces the cost of the product, As well as the production cost, therefore it is one the important methods and topics to study. Phenolic compounds are important secondary metabolites in plants that contain benzene rings with one or more hydroxyl substituent's, and range from simple phenolic molecules to highly polymerized compounds (Randhir *et al.,* 2014). However, the most common phenolic compounds, simple phenolic acids and Flavonoids, generally occur as insoluble forms (Beatriz *et al.,* 2014). It has been observed that there is a significant relation between the total Phenols and antioxidant activity as fermentation has the potential to increase the Phenols and other metabolites in various medicinal plant extracts, thus increase the antioxidant activity and might be used for treatment of human diseases in near future (Hossain *et al* ., 2019). In the light of above facts the present study is targeted to optimize biotransformation during fermentation of plant extract of *Picrorhiza kurroa* Royle ex Benth to enhance recovery of bioactive molecules such as Phenols and flavoniod.

II. MATERIAL AND METHODS

2.1 Collection of plant material:

The survey for plant collection was done in the area of Galhar village in Nagseni block in District Kishtwar of UT J&K India having GPS coordinates 33020'250N and 75055'590 E at an elevation of 1581maslKishtwar (Plate1.a&b). The rhizomes of the

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plant were collected and brought to the laboratory of Division of Microbiology, Faculty of Basic Sciences at Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha Campus for further processing.

2.2 Preparation of plant extracts:

The roots were washed under running tap water and then subjected to air drying. Dried roots (Plate.3.) were cut into small pieces and grounded into powder in a clean dried grinder. Grounded roots (Plate.3.) were extracted with distilled water and 70% Methanol solvent, separately. Powder was dissolved in distilled water with continuous shaking for at least 30 minutes and was filtered using Whatman filter paper. The process was repeated several times until the filtrate becomes colourless. For methanolic extract dried powder in methanol was kept under shaking condition for 24 hour and then filtered, this step was repeated several times till a colourless filtrate was obtained. This filtrate was than concentrated using rotary vacuum evaporator and then lyophilized (Plate.5). This dried extract was then used for further phytochemical studies.

2.3 Qualitative evaluation of plant extract:

The dried plant extract was used for studying the chemical composition of plant *Picrorhiza kurroa.* LCMS studies (Liquid Chromatography with tandem mass spectrometry) were done at IIIM Jammu. Full scan mode from m/z 100 to 1000 was performed column temperature 450C. Solvent was water with 0.1% Formic acid. Solvents were delivered at a flow rate of 0.5ml/min. The solvent was run by isocratic elution. The temperature of drying gas was 2500C, at a gas flow rate of 15L/min. HPLC column Merk RP 5µ C10 (250 × 4mm) was used for analysis. The MS spectrum was acquired in the positive ion mode (Hanafi *et al.*, 2018).

2.4 Preparation of inoculums:

Saccharomyces cerevisiae MTCC 475(Plate.5.)and *Lactobacillus acidophilus* JU (Plate.7.) were used in this study. The standard cultures were subcultured on respective media. Slants were prepared by streaking of these cells and sub culturing was done, for *Saccharomyces cerevisiae*, YPD agar (Yeast peptone dextrose) was used and for *Lactobacillus acidophilus*, MRS (*e Man, Rogosa and Sharpe*) agar was used for sub culturing, the cells were grown at 27oC (*Saccharomyces cerevisiae*) and 37oC (*Lactobacillus acidophilus*) for 24 hours then again cultured using the same broth to get initial cell count of 108 CFU/ml by using McFarland's standards by adjusting the turbidity of the microbial suspension. For 108 CFU/ml the suspension was compared to the 0.5 McFarland standards and the OD was recorded at 600nm.

2.5 Fermentation process optimization:

Fermentation was carried out using shake flasks. Classical One factor at one time method was used for studying fermentation parameters, where only one factor was kept varied while others were kept constant. For fermentation measured quantity of powdered roots get dissolved in 200ml distilled water and then sterilized at 121oC, inoculated(plate.8.) and incubated in shaking condition in BOD incubator (Plate.8.) (Nisa et *al.*, 2018). Four fermentation parameters were determined during the study i.e. temperature, incubation time, pH and inoculums load. For all factors three levels i.e. low, middle and high were evaluated of Phenols and Flavonoids. All the experiments were performed in triplets and blank was maintained simultaneously for comparison. The filtrate was then subjected to centrifugation at 10,000 rpm for 10 minutes to make the filtrate cell free; supernatant was then used for further analysis.

2.6 Determination of phenolic content:

Total phenolic content was determined using Folin Ciocalteu's phenol reagent method with certain modification as described by Chang *et al.,* 2001. In 100μl of supernatant 1ml 1N FCR was added and incubated for 5min at RT, then 3ml 20% sodium carbonate was added, again incubated for 20 min and read the absorbance at 760nm. The total phenolic content is expressed as µg/ml using gallic acid calibration curve as standard (Fig1).

2.6.1 Determination of total Flavonoids content:

TFC was determined using aluminium chloride method with modification in method as described by Tarnawski *et al.,* 2006. In 250µl of supernatant add 1.25ml distilled water and 75µl of 5% sodium nitrate and incubate for 5 min, and then add 150µl of aluminium chloride incubate for 6 min and add 1M 500µl of sodium hydroxide and 275µl distilled water mix well and read the absorbance was read at 510nm.total Flavonoid content is expressed as µg/ml using quercetin calibration curve as standard (Fig2).

III. RESULTS

3.1 Survey and collection of plant samples:

The survey for plant collection was done in Galhar, a village in Nagseni Block of District Kishtwar in UT of J&K, India, having GPS coordinates $33^{0}20'25^{0}$N and $75^{0}55'59^{0}$ E, at an elevation of 1581maslKishtwar (Plate. 1&2). Healthy plant samples were brought to the laboratory for further processing and stored at 4^{0}C in refrigerator for future studies.

3.2 Preparation of plant extract:

The plant roots were dried in oven at 60^{0}C for (Plate.3.) and grounded to powder, which was then used for further extraction (Plate.4.) Two extraction solvents were used for extraction i.e. Distilled water and 70% Methanol, where it is revealed that the aqueous extract showed more peaks i.e. 25Nos (Fig.3.) as compared to Methanolic extract i.e.15Nos only (Fig.4.). Further studies were conducted using water as solvent only as it proved better in compound recovery as compare to alcoholic solvent used.

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3.3 Preparation of inoculums:

Two microorganisms were used for the fermentation studies i.e. *Saccharomyces cerevisiae* and *Lactobacillus acidophilus* procured from IMTECH-Chandigarh and University of Jammu. Sub culturing was done on selective media i.e. YPDA (Yeast peptone dextrose agar) for *Saccharomyces* (Plate.6.) and MRS agar (Man, Rogosa, Sharpe) for *Lactobacillus acidophilus* (Plate.7.). Further studies were conducted using these two cultures in respective culture media.

3.4 Fermentation process optimization:

Fermentation studies were done by shake flask method. Fermentation parameters i.e. Temperature, Incubation time, Inoculum load and pH were optimized using classical one factor at onetime method (Plate.8&Plate.9.). Three factors were analysed for each parameter. Total phenolic and Flavonoid content was determined using FCR (Folin–Ciocâlteu reagent) and Aluminium chloride method respectively and the absorbance was recorded at 760nm for Phenols and at 510nm for Flavonoids.

In case of *S.cerevisiae*, the conditions for fermentation were incubation time- 48h, 72h and 96h, temperature -25^{0}C, 27^{0}C ,30^{0}C ,pH-4.0, 5.5, 7.0 and inoculum load-10^{4}CFU/ml, 10^{8}CFU/ml and 10^{12}CFU/ml whereas in case of *L acidophilus* , the conditions for fermentation were incubation time48h, 72h and 96h, temperature 30^{0}C, 37^{0}C and 45^{0}C pH-4.0, 6.2, 8.0 and inoculum load10^{4}CFU/ml, 10^{8}CFU/ml and 10^{12}CFU/ml.

During studies performed with *S.cerevisiae*, maximum increase in phenolic and flavonoid content was observed at 48h of incubation time where the total phenolic content has increased from 94.03mg GAE/g dw to 136.77mg GAE/g dw and total Flavonoid content from 12.16mg QE/g dw to 22.38mg QE/g dw. Further no increase was observed at 72h and 96h of incubation time, instead a decline in total phenolic content and total flavonoid content was observed where value has decreased from 136.77mg GAE/g dw to 106.75mg GAE/g dw for phenols and from 22.38mg QE/g dw to17.64mg QE/g dw for flavonoids(Table. 1a &b). In case of pH a maximum increase in phenolic and Flavonoid content was observed at pH 5.5 (92.43mg GAE/g dw to 128.96mg GAE/g dw for phenols and 11.17mg QE/g dw to 21.62mg GAE/g dw for flavonoids) (Table. 2a&b) and in case of inoculum load studies, an increase was observed at 10^{8}CFU/ml(110.31mg GAE/g dw to 192.26mg GAE/g dw for phenols and 13.75mg QE/g dw to 14.14mg QE/g dw for flavonoids)(Table.3a&b).Temperature studies have also showed an impact on the growth and enhancement of total phenols and total flavonoids. In case of *S. cerevisiae* maximum increase was observed at 30^{0}C (92.10mg GAE/g dw to 110.23mg GAE/g dw and for flavonoids from 11.38mg QE/g dw to 12.19mg QE/g dw) (Table.4a&b).

Fermentation studies performed with *L.acidophilous* has recorded a maximum increase in phenolic and Flavonoid content from 48h to 72h of incubation time i.e. 94.94mg GAE/g dw to 109.39mg GAE/g dw for phenols and 11.52mg QE/g dw to 14.21mg QE/g dw for flavonoids, followed by decline in values i.e. from 109.39mg GAE/g to 102.27mg GAE/g dw for phenols and from 12.87 mg QE/g dw to 14.02mg QE/g dw for flavonoids at 96h of incubation time (Table. 1a&b) and at pH 6.2 (92.67mg GAE/g dw to 110.04mg GAE/g dw for phenols and 10.71mg QE/g dw to 13.37mg QE/g dw for flavonoids) (Table. 2a&b). Lactobacillus as fermenting micro-organism recorded maximum enhancement of phenols (93.21mg GAE/g dw to 118.39mg GAE/g dw) and flavonoids (11.52mg QE/g dw to 14.79mg QE/g dw) at 37^{0}C (Table.4a&b) and in case of inoculum load maximum increase was observed at 10^8 CFU/ml i.e. from 101.21,g GAE/g dw to 120.22mg GAE/g dw for phenols and from 12.17mg QE/g dw to 14.13mg QE/g dw for flavonoids.

All these findings were further confirmed by qualitative analysis at IIIM Jammu by LCMS studies (Table.5, 6&7). Data revealed that water extraction medium showed maximum phytochemical recovery as compare to that of methanolic extraction (Table. 5&6).Aqueous extract showed 25 number of peaks whereas only 15 peaks were noted in methanolic extract indicating varied product formation in both solvents. A total of 11 peaks were recorded common among both the solvents indicating more solubility of water toward different metabolites that are present in plants. Based on these observations we selected water as solvent for further fermentation studies. For fermentation two microbes were used i.e. *Saccharomyces cerevisiae* and *Lactobacillus acidophilus.* Results of quantitative analysis indicate that *S. cerevisiae* had enhanced total phenols and flavonoids after 48 h of incubation whereas *L.acidophilous* showed enhance in total phenols and flavonoids till 72h of incubation period (Table. 1a & 1b). Quantitative studies have also indicated that, as compared to the control i.e. non- fermented aqueous extract; fermented water extract had more total phenolic and flavonoid content.

LCMS analysis of control samples (without micro-organism) showed varied results indicated in Table.5, 6 and 7 and Figures.3&4. Water extract had recorded 25 peaks whereas methanolic extract of plant recorded only 15 peaks.

During optimization of various fermentation parameters, studies have indicated that in control i.e. non-fermented water extract,25 peaks obtained from both 48h NF SC (Non-fermented control for *S.cerevisiae*) and 72h NF LA (Non-fermented control for *L. acidophilous*). Out of 25 peaks, four were common in both fermented and non-fermented samples. Comparison of peak area of common peaks revealed that there is an enhancement in fermented products as compared to non-fermented. Peaks at RT (retention time) 2.345 showed an increase in peak area from 7680074 to 9915541 in case of fermentation using *S.cerevisiae*, whereas it was

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recorded that *L.acidophilous* had 50, 76285 to 68, 03796 in case of *L.acidophilous*. Another peak which showed increase in peak area was at retention time 2.630 which showed increase from 8,520863to35,465673 in case of *S.cerevisiae* whereas from 22,617858 to24,628579 in *L.acidophilous*. Similarly, peak at retention time 27,508 showed a decrease in the peak area from 66, 73694-48, and 46174. All these results are in accordance with the quantitative analysis done using spectrophotometer during the study.

IV. DISCUSSION

In present study it is revealed that the phenolic content increased during the initial hours of fermentation i.e. at 48h as can be seen in Table. 1 the value has increased from 94.03 to 136.77 but as the fermentation time was increased there occurs a decrease in the phenolic content in both the cases. This trend is similar to the study reported by Alvarez *et al.*, (2017) in okra seeds where the phenolic content increased initially but later on increasing incubation time showed decrease in the content. Same trend was observed by Lasekan and Shabnam (2013). This could be due to action of polyphenoloxidase enzyme which is responsible for catalyzing the poly Phenols oxidation to high molecular weight condensed polyphenols. Naturally phenolic compounds are combined with sugar, and usually occur in esterified form linked to the cell wall matrix and as such are not readily available.

But during fermentation, proteolytic enzymes of the cells hydrolyse the complexes of phenolics into free, soluble, and other simpler and more active forms thus increasing their bioavailability. Another reason for the decrease is could be due to the diffusion of phenolics into cell liquids by polyphenol oxidase. Further maximum fermentation by *S.cerevisiae* was observed at pH 5.5, this could be directly related to the growth and metabolic processes of the cells affected by the pH range during fermentation of *Picrorhiza kurroa.* Narendranath *et al.*, (2004) find the optimum pH for *S.cerevisiae growth* can vary from 4-6 depending on the fermentation temperature and yeast strain. This range is preferred may be due to optimum pH value for the activity of plasma membrane bound proteins, including enzymes and transport proteins. The internal cellular pH of *Saccharomyces* remains constant at 5.2, to maintain a constant pH cell utilize membrane protein H^+-ATPase as a proton pump.

As many enzymes function within the yeast cell during growth and metabolism and each enzyme functions best at its optimum pH which is acidic, also when the extracellular ph deviates from optimum value, the cell needs to invest more energy to either pump in or pump out H^+ ions in order to maintain the optimal intracellular pH Narendranath *et al.*, (2001). Also if the extracellular pH deviates too much from the optimum range, it may become too difficult for the cell to maintain constant intracellular pH, and the enzymes may not function normally, and in that case the cells will not be able to grow and perform fermentation Thomas *et al.*,(2002). This is most significant explanation of decrease in phenolic content when the pH was lowered from 5.5 to 4.0. in case of *Lactobacillus* maximum fermentation was observed at pH 6.2 and minimum at lower pH i.e. 4.0. At pH 4.0 the cytoplasmic pH of the cells become more acidic and even goes below their threshold value which is between 5.8-6.5 and subsequently there occurs inhibition of cellular functions kashket *et al.*,1987 and the cells will no more able to perform fermentation. In a study by Neelakantam *et al.*, 2004 also observed a restricted growth of bacteria at low pH and found the optimal pH in the range 5.5-9.

In our study we observed the maximum fermentation where value of TPC increased from 110.31mg GAE/g dw to 192.26mg GAE/g dw (Table.3a&b) at inoculum load of 10^8 CFU/ml and thus increasing the total phenolic content and the minimum TPC at inoculum load of 10^4CFU/ml. While at 10^{12} CFU/ml the values are in close range to 10^8CFU/ml. The reason for this increase from 10^4CFU/ml to 10^8CFU/ml could be due to the presence of more cells to perform fermentation at but further increase in inoculum load had not shown the same trend. This could be due to the reason that excess inoculum will lead to competition among cells for nutrients and this cause disruption in growth. Mukhtar *et al.*, 2014 have also reported a decrease in enzyme production on increasing inoculum load. Also at higher inoculum load there is production of toxins in fermented broth due to the death of older cells, which also hampers the enzyme production and activity thus occurs a decrease in the phenolic content Carlie *et al.*, 2001.

Temperature is very sensitive parameter for *S.cerevisiae* as it showed variation in fermentation at different temperature ranges in the above Table. Maximum phenolic content was observed at 30^0C. Khoja *et al.*, 2015 also reported a max yield of bioethanol after fermentation by *Saccharomyces cerevisiae* at 30^0C. As at low temperatures the cells of yeast will flocculate at bottom and become sluggish and sleepy and will work more slowly thus increasing the fermentation time also at low temperature there occurs a decrease in the membrane permeability and fluidity of the cells which affects the activities of membrane proteins, especially those involved in nutrient uptake thus in turn affects the growth and activities of the cells Nedwell *et al.*, 1999. Further at temperature higher than optimum temperature there occurs denaturation of cells, also the cells comes under stress at higher temperature and produce increased level of H_2S which hampers their metabolic processes and sometimes they even die off due to increased permeability of the cell wall to alcohol which is toxic to the cells.

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V. SUMMARY AND CONCLUSION

The present study entitled "Optimization of fermentation parameters to enhance biotransformation based pharmacophores from plant extract of *Picrorhiza kurroa* Royle ex Benth'' was conducted at Department of Microbiology SKUAST Jammu with a view to enhance the phenolic and Flavonoid content of the plant *Picrorhiza kurroa* Royle ex Benth, and also to optimize the fermentation parameters i.e. temperature, pH, incubation time, inoculum load. The Phenols and Flavonoids have antioxidant activity and are responsible for curing many human ailments.

Picrorhiza kurroa Royle ex Benth is being used in Indian System of Medicine since ages to treat the liver related ailments. In our study two microorganisms were used for fermenting *Picrorhiza kurroa* plants i.e. *Saccharomyces cerevisiae* and *Lactobacillus acidophilus,* procured from IMTECH- Chandigarh and University of Jammu, Jammu. Two extraction mediums were used where it was observed that the aqueous extract is better over the methanolic one as it showed a greater number of compounds; therefore further fermentation studies were done on aqueous extracts. Fermentation procedures was done by using shake flasks and filtrates were used to determine the total phenolic and Flavonoid content by FCR and Aluminium chloride method respectively. From the findings of quantitative and qualitative analysis, we concluded that the phytochemical availability in plant *Picrorhiza kurroa* has increased upon fermentation using yeast and bacteria. Differences in the total phenolic and Flavonoid content was observed on changing the fermentation parameters viz. temperature, pH, inoculum load and incubation time.

This gave an idea about the ideal conditions for the fermentation of plant and this information can be used in the experiments of enhancing the bioactive components of the plant. Further many studies have reported the same trends as we observed during our experimental work. This study also concluded that the *Saccharomyces* was better for fermentation of *Picrorhiza kurroa* Royle ex Benth over *Lactobacillus*. Bioactive components showed maximum increase at 30^0C temperature with pH 5.5. Also, it was seen that increasing incubation time had a positive effect on total phenolic content and Flavonoid content but prolonged fermentation would result in the decrease in both phenolic and Flavonoid content. In case of *Saccharomyces,* a decrease was observed after 48h but in case of *Lactobacillus* a decrease was seen after 72hrs of incubation indicating that yeast has the better ability to ferment.During fermentation microbes release variety of enzymes; thereby causing cleavage and acidification of glycosides and esters linked to phenolic acids and thus releasing the bound Phenols into free Phenols consequently this improves the nutraceutical value and increase the bioavailability due to improvement in free phenolics.

Picrorhiza kurroa is a herb that has various bioactive compounds which have medicinal use, present study have reported the enhancement of these bioactive compounds (Phenols and Flavonoids) upon fermentation, further different fermentation conditions have varied effects on these compounds. From our study it is revealed that fermentation by *Saccharomyces* showed more efficient increase in phenolic content as compared to *Lactobacillus.* Thus, this study gives an idea about optimum fermentation parameters and microorganism for fermentation and to enhance bioactive compounds in *Picrorhiza kurroa* Royle ex Benth.

Following are the conclusion drawn from this novel study:

- This study concluded that *Picrorhiza kurroa* Royle ex Benth is present naturally in Galhar area situated at an elevation of 1581 meters, with $33^020'25^0$N and $75^055'59^0$ E coordinates of District Kishtwar in Jammu Division.
- *Picrorhiza kurroa* has various Phenols and Flavonoids as one of its bioactive compounds responsible for its medicinal properties.
- From the two extraction solvents aqueous extract shows more availability of compounds over methanolic one.
- Quantitative analysis revealed the enhancement of phenolic and Flavonoid compounds after fermentation, where the maximum enhancement was observed at temperature 30^0C and pH 5.5 at 48h in case of *Saccharomyces cerevisiae and* 37^0CandpH 6.2 at 72h, in case of *Lactobacillus acidophilus*.
- From quantitative and qualitative analysis *Saccharomyces cerevisiae* proved to be better for fermentation, as on average 49.30% increase in phenolic and Flavonoid content was observed whereas in case of *Lactobacillus acidophilus* it was only17.60%.

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