STUDY OF POTENTIAL COMPOUNDS OF FRESH AND DRIED BERRIES AND COMPARATIVE ANALYSIS OF THEIR PHENOLIC, FLAVONOID CONTENT, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES

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Abstract: There has been an increasing demand for the consumption of fruits, especially berries because of their nutraceutical value. Berries contain important bioactive compounds (BAC), which provide significant health benefits due to their antioxidant and antimicrobial effects. In this study, six different fresh and dried forms of berries were used to determine their phenolic, flavonoid content along with their antioxidant and antimicrobial potential. At first, the methanolic extraction of phenolic compounds was carried out and the extracts were further analysed. The total phenolic content was determined using Folin-Ciocalteu method and flavonoid content using aluminium chloride colorimetric method. Fresh blueberries extracts showed excellent results with 670.76µg/ml phenolic content and 27.27µg/ml flavonoid content. Antioxidant potential with value of 50.63µg/ml was observed highest in gooseberries extract, which was determined using FRAP assay. Antimicrobial activity of extracts showed maximum 37mm (fresh) and 32mm (dried) of zone of inhibition with gooseberries extract against *Staphylococcus aureus*. A comparative data of fresh and dried berries extracts was made, which showed fresh berries have higher activities in all parameters than dried berries extracts and thus, consumption of fresh berries are more fruitful. The use of berries which are an important source of BAC provides protection against harmful diseases. The phenolic compounds of berries are of great interest to nutritionist and food technologist due to the opportunity to use BACs as functional food ingredients.

Index terms- **Bioactive compounds (BAC), flavanoid, phenolic, Folin-Ciocalteu, FRAP, antioxidant**

I. INTRODUCTION

Fruits are a rich source of nutritive compounds such as vitamins, minerals, sugars, essential oils, and therefore their consumption has been increased in recent times. The bioactive compounds present in fruit juices have potent antioxidant, anticancer, anti mutagenic, antimicrobial, anti-inflammatory, and anti neurodegenerative, properties. Among fruits, berries are consumed in the largest proportion because they are delicious and provide significant health benefits. Berries belonging to different families such as Rosaceae (strawberry, raspberry, blackberry), Ericaceae (blueberry), Vitaceae (black and green grapes) are great source of BAC (bioactive compounds) (Skrovankova et al., 2015). The BAC in berries mainly contains phenolic compounds such as phenolic acids (hydroxybenzoic acid, hydroxycinnamic acid), flavonoids (anthocyanins flavonols, flavanols), stillbenes, tannins and lignans. Some berries such as strawberries, have been identified as potential sources of gallic acid and ellagic acid which have potential cancer chemopreventive activity. Anthocyanins are responsible for the characteristic colours of berries and act as a powerful antioxidant. Quercetin and catechins are abundant phytochemicals which act as a potent antioxidant that has additional important biologic, pharmacologic, and medicinal properties (Puupponen-Pimiä et al, 2005, Phuyal et al., 2020). Antioxidants helps in protecting the body against harmful superoxide radicals which are associated with aging process and provides benefit in improving quality of life by preventing or postponing the onset of degenerative diseases (Skrovankova et al., 2015). The presence of phenols and flavonoid in high amount in extracts of berries such as blueberries, strawberries, and gooseberries are responsible for their high scavenging activity towards superoxide radicals (Lacombe et al., 2017). Their antioxidant activity is related with the number of hydroxyl groups present in molecular structure of phenolic compounds (Diaconeasa et al., 2014). The growing demand of berry-derived supplements leads to study interaction of berries with microorganism because the enzymatic reactions occur with phenolics after consumption can affect human health. Research has demonstrated that berries have antimicrobial properties, with consensus that inhibitory effects come from their antioxidant compounds (Lacombe et al., 2017). Phenolic compounds shows their antimicrobial effect by interfering with energy transduction process, where they bind with special components of electron transfer chain and inhibit electron flow, imbalance the proton gradient, and prevent ATP synthesis.

The study aims to carry out extraction of phenolic compounds from fresh and dried berries and to determine their total phenolic, flavonoid content, antioxidant and antimicrobial potential along with comparative analysis between fresh and dried berries.

II. RESEARCH METHODOLOGY

2.1 Collection of samples

Fresh samples of blueberries, strawberries, gooseberries, black grapes, green grape, and Indian jujube (wild berries), were purchased from the market during the growing season in the month of February-March from Valsad district.

2.2 Processing of samples

The fresh berry samples were washed and cleaned thoroughly, and were stored in a freezer at -20°C until they were analyzed. For the dried form of berries, freshly purchased berries were clean and sun-dried for a week before the extraction process. Fresh berries samples were first grinded with the help of mortar and pestle. The dried powdered form of samples was made by grinding sun-dried berries in a mixture grinder and was then packed in an air-tight container before use.

2.3 Extraction process

Methanolic extraction process was used to extract the phenolic compounds from berries and seeds. A known amount of samples (6 gm) were weighed and were extracted with 80% aqueous methanol (15 ml). The mixture was then incubated on an orbital Shaker at 70°C for 2 to 3 hours. After incubation, the mixture was centrifuged at 2500rpm for 15 mins. Then it is filtered using the filter paper on a Buchner funnel and the filtrate was then assayed for Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Antioxidant and Antimicrobial activity.

2.4 Determination of Total Phenolic Content (TPC) of berries

Various concentrations / dilutions of extracts were prepared using distilled water (1:2, 1:5, 1:10, undiluted). 1 ml of extract of each concentration was added to the test tube. Then to each concentration, 0.5 ml of folin-ciocalteu reagent was added and the mixture is then incubated in dark for 10 minutes. After incubation, 2 ml of 20% sodium carbonate was added to the mixture and was then incubated for 30 minutes in dark. Then the absorbance was measured at 700 nm against blank using colorimeter. The slope of the standard calibration curve of gallic acid was used to determine the concentration of the total phenolic content present in the extracts. Then, the average of concentrations was calculated that determine the total phenolic content in µg/ml present in the sample. The following formula was used to calculate the concentration of phenolic content:

$$\text{Concentration (µg/ml)} = \frac{\text{Absorbance at 700 nm}}{\text{slope of the calibration curve}}$$

2.5 Determination of Total Flavonoid Content (TFC) of berries

Various concentrations/ dilutions (1:2, 1:5, 1:10, undiluted) of extracts were prepared. 1 ml of extract of each concentration was added to the test tube along with 1 ml of distilled water which is followed by the addition of 0.2 ml of 5% sodium nitrate to the mixture. The mixture is then incubated at room temperature for 6 minutes. Then, 0.3 ml of 10% aluminium chloride solution was added to the mixture which is then incubated for 5 minutes at room temperature. Then, 0.4 ml of 1M sodium hydroxide was added to the mixture and the solution is mixed well and the absorbance was measured at 510 nm against blank. Using the slope of the standard quercetin calibration curve, concentrations of total phenolic content were calculated. The average of concentrations signifies the total phenolic content of the extract in µg/ml. The following formula was used to determine the concentration:

$$\text{Concentration (µg/ml)} = \frac{\text{Absorbance at 510 nm}}{\text{slope of the calibration curve}}$$

2.6 Determination of antioxidant activity

The antioxidant activity of berries and seed extracts was determined using a FRAP (ferric reducing antioxidant potential) assay. It is a convenient rapid screening method for measuring the antioxidant potential. 1 ml of extract was mixed with 1 ml of 0.2 M phosphate buffer and 1 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 °C for 20 minutes. After incubation, 1 ml of 10% trichloroacetic acid was added to the mixture. Then the mixture was centrifuged for 10 minutes at 3000 rpm. The upper layer (1 ml) was then combined with 0.5 ml of 0.1 % ferric chloride and was mixed. The absorbance was then measured at 700 nm. A standard ferrous ammonium sulfate calibration curve was used for the determination of antioxidant concentration and following formula was used to calculate the concentration.

$$\text{Concentration (µg/ml)} = \frac{\text{Absorbance at 700 nm}}{\text{slope of the calibration curve}}$$

2.7 Determination of antimicrobial activity

The Agar well diffusion Assay was used to determine the antimicrobial activity of extracts.

Bacterial strains and culture conditions

The test organisms were procured from the laboratory and were tested for their purity by performing Gram's staining. The organisms that were used as test organisms to study the antimicrobial activity of berries are as follows: *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Saccharomyces cerevisiae*. Frozen stock cultures of test organism were used which were maintained at -20°C. Before the experimental use, cultures were transferred on a solid media and incubated for 24-48 hours. Cultures were then sub-cultured in liquid media, then incubated for 24- 48 hours, and were then used as the source of inoculum for each experiment. For bacterial cultures, nutrient agar slant and nutrient broth were used and for yeast, glucose yeast extract agar (GYE) slant and glucose yeast extract agar (GYE) broth were used. The concentration of microbial inoculum was set in the range of 10^6CFU/ml (0.5 according to the Mcfarland chart).

Agar well diffusion assay

Nutrient agar plates were used for the assay. The microbial culture was streaked on the nutrient agar plates with the help of a sterile cotton swab to obtain the lawn growth of the organism. Then, with the help of a cork borer (6mm) well was made in the center of the plate. An appropriate aliquot of extract (1 ml) was then added to the well with the help of a sterile pipette. The plates were then incubated at 4-5 °C in refrigerator for 30 minutes for proper diffusion of the extract. Then the plates were incubated at 37° C for 24-48 hours. After incubation, the zone of inhibition was measured.

III. RESULTS AND DISCUSSION

3.1 Sample Pretreatment

For dry sample of berries, the fresh samples were washed properly and were kept in a clean tray and all the samples were sun-dried for a week. The dried samples were then grinded in a mixture grinder, and the milled powder of sample was packed in an air tight container before analysis. As shown in fig.1, the samples were made into crushed and powdered form before analysis.

Figure 1: Grinded samples of fresh berries

3.2 Results of Extraction Process

The fig.2 shows the extraction of phenolic compounds using methanol as solvent.

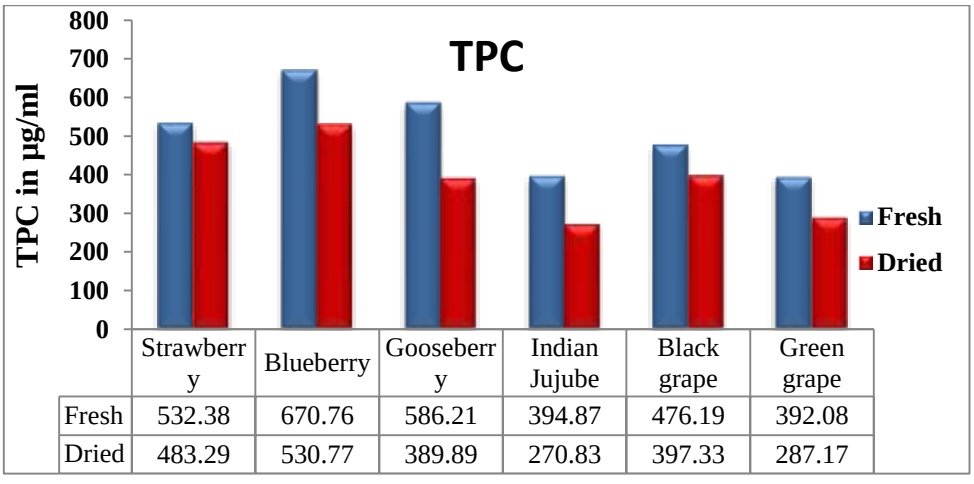
Figure 2: Methanolic extracts of fresh and dried berries

3.3 Total Phenolic Content (TPC) of berries

Total Phenolic Content of six different fresh and dried forms of berries extracts were determined using Folin-Ciocalteu method. It is based on the formation of blue complex compounds due to the reaction between the phenolic compounds and the Folin-Ciocalteu reagent which can be measured at a maximum wavelength. In the current study, the TPC value ranges from 70µg/ml to 700µg/ml.

Comparative analysis of fresh and dried berry extracts

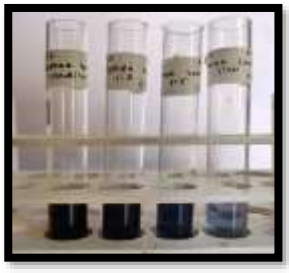
The comparative study of six different fresh and dried berries extracts are represented in the fig.3. The TPC values were higher in fresh berry extracts than in dried extracts. The TPC value of fresh blueberry was observed highest of about 670.76µg/ml and dried form showed value of 530.77µg/ml. The lowest value of TPC was observed in Indian Jujube (wild berry) of 270.83µg/ml and its fresh form had value of 394.87µg/ml. Table 1 represents the results of comparative analysis of TPC between fresh and dried gooseberry and blueberry.



	Strawberry	Blueberry	Gooseberry	Indian Jujube	Black grape	Green grape
Fresh	532.38	670.76	586.21	394.87	476.19	392.08
Dried	483.29	530.77	389.89	270.83	397.33	287.17

Figure 3: Comparative analysis of Total Phenolic content of fresh and dried berries extract

Table 1: Results of comparative analysis of Total Phenolic content of fresh and dried berries extracts.

Berry Extract	Fresh	Dried
Gooseberry		
Blueberry		

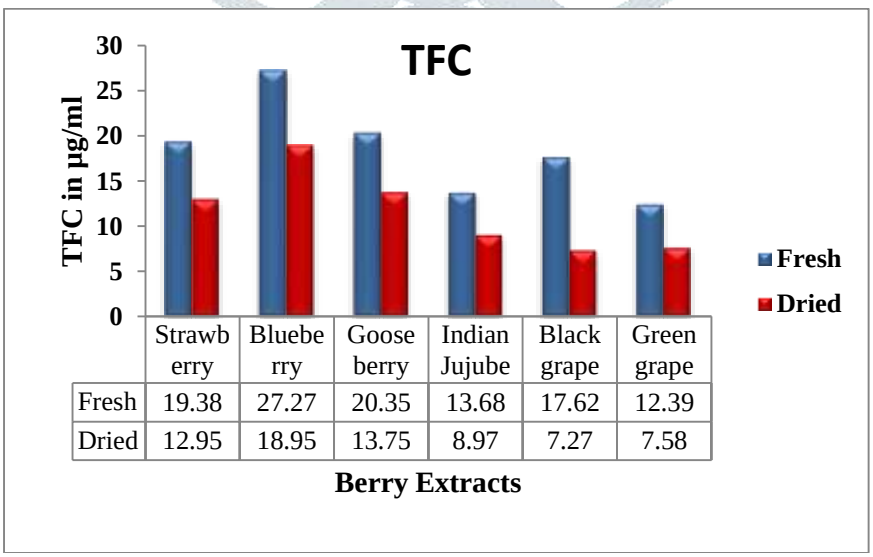
3.4 Total Flavonoid Content (TFC) of berries.

The TFC has been investigated using aluminium chloride colorimetric method, based on the formation of complex between aluminium ions and the carbonyl and hydroxyl groups present in the flavonoids. Six berries extracts were analysed and different concentrations of flavonoid content were reported. The present study has range of TFC between 0µg/ml to 30µg/ml.

Comparative analysis of fresh and dried berry extracts

As phenolic components are directly related to flavanoids present in extracts, the TFC of fresh berry extracts are also higher than dried berries, which is represented in fig.4. The highest TFC value of 27.27µg/ml was observed in fresh blueberries extract followed by 20.35µg/ml in fresh gooseberries, and 19.38µg/ml in fresh strawberries. Dried black grapes have the lowest flavanoid content with TFC value of 7.27µg/ml have been observed. Figure 5 represents the results of TFC in fresh and dried berries.

Blueberry are rich in flavonols such as myricetin glycoside, quercetin glycosides and anthocyanins such as cyanidin glycosides, delphinidin glycosides, malvidin glycosides which are responsible for high flavonoid content (Skrovankova et al., 2015). The high TFC content of berries are better for consumption as they have pharmacological impact and has anti-inflammatory and anti-diabetic properties.



	Strawberry	Blueberry	Gooseberry	Indian Jujube	Black grape	Green grape
Fresh	19.38	27.27	20.35	13.68	17.62	12.39
Dried	12.95	18.95	13.75	8.97	7.27	7.58

Figure 4: Comparative analysis of Total Flavonoid content of fresh and dried berries extracts.

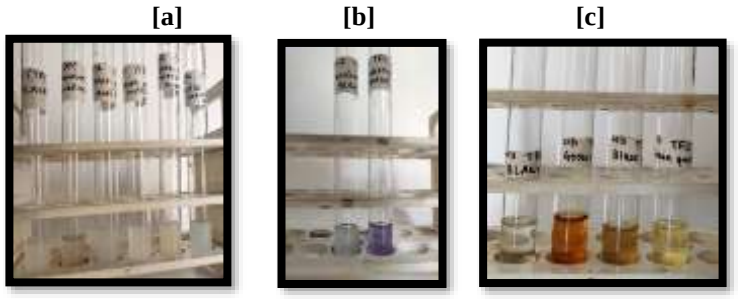


Figure 5: Results of Total Flavonoid content (a) Results of TFC of fresh berry extracts, (b) Results of TFC of fresh and dried berry extract, (c) Results of TFC of dried berry extracts.

Gooseberries have the presence of polyphenols such as flavonoids, kaempferol, ellagic acid and gallic acid along with punicafolin and phyllanemblinin A, which are majorly responsible for high flavonoid content in it (Habib-ur-Rehman et al.,2007).The reason for reduced TFC in dried sample may be due to sun exposure which results in denaturation of BAC in extracts.

3.5 Antioxidant Potential of Phenolic Extracts

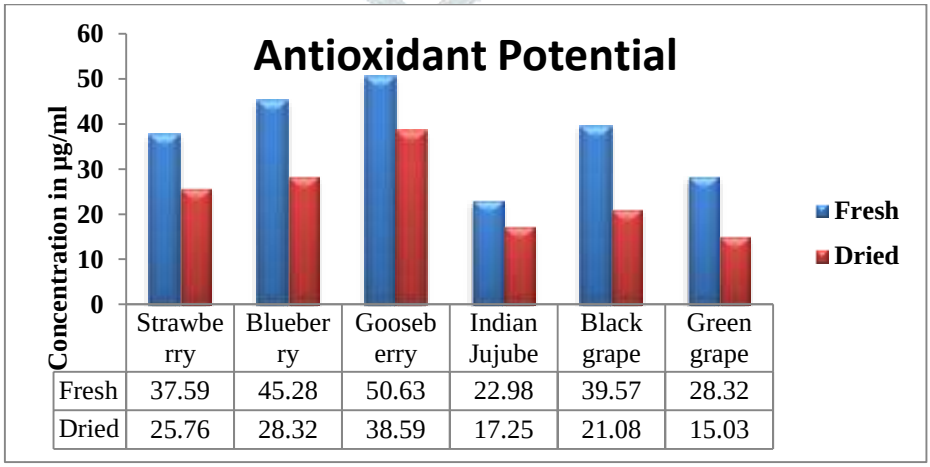
Antioxidants have scavenging properties. Antioxidants are associated with the phenolic content present in the extract. In present study, antioxidant potential was estimated using FRAP assay, which is based on reduction of ferric (Fe^{3+}) complex to ferrous (Fe^{2+}) complex. According to the data obtained from analysis, antioxidant potential berry extracts ranges from 0µg/ml to 50µg/ml.

Comparative analysis of fresh and dried berries extracts

As with TPC and TFC, antioxidant potential of fresh berry extracts are higher than dried sample extracts, shown in fig.7. With value of 50.63µg/ml, fresh gooseberries extract have the highest antioxidant potential and its dried form with 38.59µg/ml potential was observed, which was also higher than most other berries. Dried Indian jujube and green grape extracts showed lowest values of 17.25µg/ml and 15.03µg/ml respectively.The results are represented in fig. 6.

The high antioxidant potential of gooseberries is due to the presence of ellagitannins such as emblicanin A, emblicanin B and high amounts of ascorbic acid (Tarwadi K et al., 2007, Dharmananda S, 2003). The antioxidants in fruits provide protection against cancer and heart diseases. Resveratrol, a compound of stilbene is widely found in varieties of berries which are majorly responsible for antioxidant potential and it helps to fight aginst colon cancer and breast cancer.

Figure 6: Results of antioxidant activity of fresh and dried berries extracts.



	Strawberry	Blueberry	Gooseberry	Indian Jujube	Black grape	Green grape
Fresh	37.59	45.28	50.63	22.98	39.57	28.32
Dried	25.76	28.32	38.59	17.25	21.08	15.03

Figure 7: Comparative analysis of antioxidant potential of fresh and dried berries

3.6 Antimicrobial Potential of Phenolic Extracts

Antimicrobial activities of six fresh and dried berries were analysed using Agar well diffusion assay, which is suitable for semi-quantitative estimation. It is based on the diffusion of potent compound into the medium that inhibits the growth of test

organism and gives clear zone of inhibition. The obtained results showed that the methanolic extracts of berries strongly inhibited the growth of test organisms and clear zones of inhibition were observed.

Comparative analysis of fresh and dried berries extracts

The results represented in the table 2 shows that sensitivity of phenolic extracts vary significantly among different berries. Both dried and fresh extracts strongly inhibited the growth of selective organisms, but comparative analysis showed fresh berries showed higher antimicrobial activity than dried forms, this may be due to the BAC inactivation because of sun exposure. The high amount of chlorogenic acid in gooseberry showed highest inhibition zone against *Staphylococcus aureus,* of 37mm in fresh extract and 32mm in dried extract. Blueberries extract is rich in ascorbic acid, which gave zone of 27mm in fresh berry extract and 25mm in dried berry extract against *Staphylococcus aureus.* The best results of comparative analysis between fresh and dried berries are given in table 3 and table 4. According to the results of table 2, Gram positive organisms are seen more sensitive to berry phenolics as compared to Gram negative. This may be due to the presence of lipopolysaccharide, outer layer in Gram negative organism which act as a permeability barrier and is responsible for the intrinsic resistance of these organisms to antimicrobial compounds. It is also observed that *Saccharomyces cerevisiae* were more resistant than any other selected organism. But, it was inhibited by fresh blueberries extract with a zone of 21mm. Other organisms such as *Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli* are readily inhibited by strawberries extracts, black and green grapes extracts. Fresh black grape extract shows the maximum inhibition zone of 31mm against *Pseudomonas aeruginosa* and zone of 30mm against *Escherichia coli*.

Table 2: Comparative analysis of antimicrobial activity between fresh and dried berries extracts.

	Zone of Inhibition in mm after 24 hours											
Berry	**Strawberry**		**Blueberry**		**Gooseberry**		**Indian Jujube**		**Black Grape**		**Green Grape**	
Organisms	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried
Bacillus cereus	**20**	**15**	**27**	**22**	**30**	**26**	**21**	**16**	**33**	**22**	**34**	**20**
Staphylococcus aureus	**30**	**13**	**27**	**25**	**37**	**32**	**25**	**13**	**27**	**18**	**25**	**17**
Pseudomonas aeruginosa	**16**	**13**	**15**	**14**	**19**	**16**	**14**	**12**	**31**	**14**	**32**	**14**
Escherichia coli	**13**	**11**	**13**	**11**	**27**	**19**	**12**	**12**	**30**	**24**	**32**	**24**
Saccharomyces cerevisiae	**12**	**11**	**21**	**19**	**19**	**18**	**13**	**11**	**13**	**11**	**19**	**13**

Table 3: Results of comparative analysis of antimicrobial activity between fresh and dried gooseberries extracts.

Organisms	**Gooseberries Extracts**	
	Fresh	**Dried**
Staphylococcus aureus		
Pseudomonas aeruginosa		

Saccharomyces cerevisiae		

Table 4: Results of comparative analysis of antimicrobial activity between fresh and dried blueberries extracts.

Organisms	**Blueberries Extracts**	
	Fresh	**Dried**
Bacillus cereus		
Escherichia coli		
Saccharomyces cerevisiae		

IV. CONCLUSION

The present study investigated total phenolic, flavonoid contents, antioxidant, and antimicrobial properties of fresh and dried berries and they were found considerably good. However, these parameters were remarkably better in fresh berries extracts as compared to dried berries extracts. The results of current study concludes that consumption of fresh berries are more fruitful than dried berries. The variations in phenolic compounds among berries is due to the difference in chemical constituents and also due to the cultivar, growing location, environmental conditions, ripening stage, harvesting time and storage and processing of berries. The results of the present study showed that blueberries and gooseberries could be a potent source of bioactive compounds because of their high activity. The antimicrobial activity of berry extracts shows an effective zone of inhibition again selected organisms such as *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli,* and *Saccharomyces cerevisiae* which can easily contaminate the food products. These plants having potent BAC (Bioactive compounds) could be of greater importance in preventing harmful human diseases. Antimicrobial activities of berries phenolic extracts leads to several new applications as inhibitory agents in food industry and medicine. Further investigations are required to use berry phenilocs with traditional medicine along with mechanism of action of phenolics on beneficial organism.

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