Parabens are naturally occurring phenolic compounds commonly found in fruits such as blueberries and are valued for their antimicrobial and preservative properties. This study aims to extract parabens from blueberries and purify them using column chromatography. The extraction was carried out using a methanol-water solvent system (80:20 v/v) to maximize the yield of parabens. The crude extract was filtered and concentrated before further purification. Solvent partitioning with ethyl acetate was performed to enhance the separation of parabens from other non-polar compounds. The concentrated extract was subjected to column chromatography using silica gel as the stationary phase and a hexane-ethyl acetate gradient system as the mobile phase. Fractions were collected systematically and analyzed for the presence of parabens using Thin Layer Chromatography (TLC), with standard parabens as reference markers. Selected fractions containing parabens were further validated using High Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS) for confirmation and quantification. The results demonstrated successful extraction and isolation of parabens from blueberries, with efficient separation achieved using column chromatography. The findings can contribute to the development of natural preservatives for use in pharmaceutical, cosmetic, and food industries, offering an eco-friendly alternative to synthetic parabens.

**Introduction**

Blueberry (Vaccinium spp.) belongs to the family Ericaceae, subfamily Vaccinoideae, genus Vaccinium. It is popular because of its fresh and sweet fruit and rich nutritional value. Recently, the blueberry was called a “super fruit” by the Food and Agriculture Organization of the United Nations (FAO). It is one of the five healthiest fruits in the world. In recent years, blueberry production and consumption have increased with significant economic value. Blueberries are classified into four species based on morphological classification: rabbit-eye blueberry (V. virgatum), northern high shrub (V. corymbosum), southern high shrub (V. formosum), and low shrub (V. angustifolium) [1].

BLUEBERRY (Vaccinium spp. Family; Ericaceae) is a potential berry fruit for acidic soils. There are several types of blueberries like: low bush (Vaccinium an gustifolium), high bush (Vaccinium corymbosum) and bilberry or forest blueberry (Vaccinium myrtillus) etc. High bush blueberry is suitable for cultivations and most widely cultivated, other species of minor importance are Vashei, the rabbit eye blueberry, which is native to the South-Eastern United States, and the low chill southern high bush, derived from northern high bush types crossed with several species such as V. darrowi and V. shei are proven suitable in warm climates with low winter chill. The cultivated (high bush) blueberry is a bush generally grows up to 6 to 12 feet (1.8 to 3.6 m) height with simple, elliptic to ovate deciduous leaves. The leaves are slightly waxy above with pubescence at least on the veins beneath. The trunk may be single or multiple sometimes numerous
suckers arises near main trunk [2]. The USA is the leading blueberry producer, due to its ideal growing climate with abundant land while Chile is main supplier of off-season blueberry in world. Other countries in the southern hemisphere are: Australia, New Zealand and South Africa. According to FAO, the annual production of blueberries in world is 3,11,959 million tonne from 72,306 ha area. In Europe, Blueberries are being grown in Germany, the Netherlands, Poland, Italy and Hungary. Besides many growers in France, Austria, and Italy are growing it on commercial-scale. Even in Belgium and Norway, some very promising trials with special methods of Blueberry cultivation (heated greenhouse production) resulted in a limited commercial production which is successful. Its commercial cultivation was not reported in India but in Himachal Pradesh at CSK KVV, Palampur Blueberry was introduced in 2007-08 and showed adaptive to this climate since the soil here is slightly acidic and climate during winter remains cool enough to meet out chilling requirement up to some extent.3 Most parts of the blueberry plant can be used, including the fruit, leaves, and stems. The fruit is consumed raw or processed into juice, jam, wine, etc.; leaves and stems are the alternative source of bioactive natural products and its distinctive color is often used as natural dye. Moreover, because of its rich nutritional content, the blueberry has been developed into many nutritional supplements for disease prevention and dietary regulation, such as blueberry lutein eye care tablets, blueberry dietary supplements (such as blueberry powder), pterostilbene capsules, etc. Blueberry fruit is greatly appreciated for its nutritional value of enriched organic acids (citric, ascorbic, phenolic acids, and tannins), phenolic compounds (stilbenoids, tannins, and flavonoid compounds, including anthocyanin, flavanone, flavanol, and quercetin), sugar, minerals, vitamins, fibers, and pectins. Phenolic compounds are divided into endogenous and exogenous phenols as per their source, and those present in plants are called endogenous phenols. By chemical phenolic compounds can be classified into tannins, flavonoids, stilbene compounds and phenolic acids. Blueberries are rich in endogenous phenols, which are the most beneficial components in blueberries and include anthocyanins, tannins, pterostilbene and phenolic acids. It shows the specific types of phenolic compounds in blueberries. Phenolic compounds are utilized as natural colorants and preservatives in foods. Studies have shown that synergies between phenolic compounds in blueberries may be responsible for their high oxidation potential, and they can thus be employed as nutritional supplements. A daily intake of blueberries (about 1/3 cup) can prevent the onset of chronic diseases, such as obesity, diabetes, atherosclerosis, etc. This review summarizes the extraction, purification, and detection techniques of phenolic compounds in blueberries [1].

**Fig 1: Blueberry Varieties and Products [4]**

**Blueberry Varieties:**
Rabbity: A southern variety known for its large size and late ripening.
High bush: A popular variety with large berries and good flavor, often used in fresh eating and baking.
Half-highbush: A cross between highbush and lowbush varieties, offering a balance of size and hardness.
Lowbush: Smaller berries with a more intense flavor, commonly used in jams and preserves.

**Products**

- **Food Products:** smoothies, jams and jellies, sauces and syrups.
- **Health Care Products:** Eye health supplements, antioxidant supplements, brain health supplements.

**Objective**

To extract parabens from blueberry samples using suitable solvent extraction techniques.

To separate parabens from other plant constituents using column chromatography.

To identify and confirm the presence of parabens in the purified fractions using analytical techniques such as Thin-Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), or Gas Chromatography-Mass Spectrometry (GC-MS).

To study the yield and efficiency of the extraction and separation process.

To investigate the potential applications of the isolated parabens in pharmaceutical, cosmetic, or food industries.

**Parabens**

Alkyl esters of p-hydroxybenzoic acid, also known as parabens, are widely used as antimicrobial agents in food products, pharmaceutical preparations, and cosmetic and toiletries consumer products. Propylparaben, n-butylparaben, iso-butylparaben and benzyl paraben, and their log Dow, PKa and aqueous solubility. It had been found that antimicrobial activity increased as the chain length of the ester group of parabens increased. However, esters of longer alkyl chains are of limited applications due to their lower solubility in water. To reach a satisfied activity, parabens are usually used as mixtures according to their antibacterial synergistic effect. Among all parabens, methylparaben and propylparaben are often used together.

Parabens have been added to food for a very long time, and the use of parabens has steadily increased in many more food categories over the years. They are employed in several foods including processed vegetables, baked goods, fats and oils, seasonings, sugar substitutes, coffee extracts, fruit juices, pickles, sauces, soft drinks and frozen dairy products at concentrations between 450 and 2000 mg kg−1.

**Extraction**

Extraction is the method of removing active constituents from a solid or liquid by means of liquid solvent. The
separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents.

**Types of Extraction**
Extraction is a process used to separate an organic chemical compound into one or more pure compounds, and it’s done using a chemical solvent known as the menstruum.

Certain pharmaceutical preparations are prepared by extraction, that is, by the withdrawal of desired constituents from crude drugs through the use of selective solvents in which the desired constituents are soluble.

**Fig 3: Methods of extraction**
**Extraction process**

**Fig 4: Extraction process**

**Definitions Involved In Process**

**Mixing**
Mixing may be defined as an operation in which each particle of any one ingredient lies as close as possible to the adjacent particles of the other ingredient. Mixing is defined as a process that tends to result in a randomization of dissimilar particles within a system.

**MILLING**
Milling is type of machining process that uses a rotating cutter to remove material in a controlled manner from a workpiece. This subtractive manufacturing technique aims to turn the workpiece into the required shape.

**TYPES**
Column & knee type
Table type milling machine
Planer type milling machine
Special type milling machine.

**Sonication**
Sonication is defined as the process in which sound waves are used to agitate the particles in the solution. These disruptions are used for mixing of the solutions, to increase the speed of dissolution of a solid into a liquid.

**EVAPORATION**
Evaporation is a physical process that turns a liquid into a gas or vapor without reaching its boiling point. It’s a natural process that happens when liquid molecules at the surface gain enough energy to escape the liquid mass. Evaporation is a key part of the water cycle and is responsible for most of the water vapor in the atmosphere.

**Re-extraction**
Re-extraction is the process of repeating the extraction of a substance in a single direction. However, the term is considered obsolete and ambiguous because the prefix “re” can mean both “back” and “again”. It should not be used as a synonym for stripping or back-extraction.

**Chromatography**
Chromatography is defined as it is a physical method of separation into its individual components when the mixture is distributed between two phases one is fixed phase called stationary phase and other is movable phase called mobile phase.

**Importance Of Chromatography**
A number of methods are available for the separation of organic and other compounds from the mixture and they are

(a) Fractional distillation
(b) Extraction
(c) counter-current distribution
(d) Crystallization

**Principle**
Components of the mixture are having different affinity Le different distribution coefficient or partition coefficient towards stationary and mobile phase, on the basis of which separation of mature can be achieved. This is the basic principle of column chromatography in column chromatography, the column is packed with stationary phase and the mixture to be separated is dissolved in a suitable solvent and allowed to pass through the adsorbent column.
Fig5: Principle of Adsorption column chromatography
Ion exchange column chromatography: It is a type of chromatography in which ion exchange resin is always used as the stationary phase.

Fig 6: Ion Exchanging Chromatography
APPLICATIONS:
Column chromatography is a versatile technique used to separate, isolate, and purify various compounds, including parabens. Parabens, widely used as preservatives in cosmetics, pharmaceuticals, and food, often require purification and analysis to ensure safety and quality control. Here are some key applications of separating parabens by column chromatography:

1. Purity Testing and Quality Control: Pharmaceutical and cosmetic industries use column chromatography to isolate parabens and assess their purity. This ensures the products meet regulatory standards and do not contain impurities that could affect safety.

2. Analysis of Complex Mixtures: Parabens are often used in combination with other preservatives and ingredients. Column chromatography helps separate them from complex mixtures, enabling more accurate analysis and quality control in finished products.

3. Environmental and Biological Monitoring: Parabens can be found in environmental samples (water, soil) and biological samples (urine, blood). Column chromatography is used to separate and quantify parabens from these samples to assess their impact on health and the environment.

4. Research and Development: In R&D, scientists use column chromatography to study the stability, efficacy, and interactions of parabens with other compounds, facilitating the development of new products and formulations.

CONCLUSION
The extraction and separation process outlined successfully isolates paraben compounds from blueberries using ethanol as the extraction solvent and silica gel-based column chromatography for purification. The use of a gradient elution method ensures effective separation of parabens from other organic compounds present in the blueberry extract. This method demonstrates the viability of using natural sources for obtaining bioactive compounds, offering an eco-friendly approach for analytical and industrial applications. Further refinements, such as optimizing solvent systems or using advanced detection methods, can enhance the yield and purity of parabens. Overall, this process serves as a reliable and efficient technique for natural product isolation.

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