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SPECTROSCOPIC AND HPTLC STUDY FOR QUALITATIVE AND QUANTITATIVE STUDY OF ALKALOID IN EXTRACT OF *EMBELIA RIBES*

Sumit Savita*, Dr. Avinash Krishnarao Kondalkar, Shankar Singh, Narendra Kumar Singh

Sun Institute of Pharmaceutical Education and Research (SIPER), Lahar, Bhind, (M.P)

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ABSTRACT-This research delves into the comprehensive analysis of alkaloids present in the extract of *Embeliaribes* using both spectroscopic and High-Performance Thin-Layer Chromatography (HPTLC) methods. The study begins with the extraction process, wherein the ethanolic extract of *Embeliaribes* yielded a significant 9.6% (w/w), highlighting the effectiveness of the extraction method employed. The phytochemical screening of the extract revealed the presence of several bioactive compounds, including alkaloids, glycosides, phenols, proteins, and tannins. In contrast, flavonoids, diterpenes, carbohydrates, and saponins were not detected, suggesting a selective presence of phytochemicals in the ethanolic extract.

Quantitative assessment was conducted to determine the total alkaloid and phenolic contents of the extract. The total alkaloid content was found to be 0.836 mg per 100 mg of dried extract, while the total phenol content was 0.474 mg per 100 mg of dried extract. These values underscore the extract's potential as a source of pharmacologically active compounds. Furthermore, HPTLC analysis was performed to quantify Embelin, a prominent alkaloid in the extract. The results indicated that Embelin constituted 0.65% of the hydroalcoholic extract. This precise quantification of Embelin, alongside the phytochemical profile, provides a deeper understanding of the chemical composition of *Embeliaribes.* The study's findings contribute to the broader knowledge of this plant's medicinal properties and its potential application in therapeutic formulations.

KEYWORDS:- *Embeliaribes,* alkaloids, phytochemical screening, High-Performance Thin- Layer Chromatography (HPTLC), spectroscopic analysis,

Corresponding author: Sumit Savita
E mail: shankarbhaskar77@gmail.com

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INTRODUCTION: -Spectroscopy is the study of interaction of electromagnetic radiation with matter. These interactions involve absorption and emission of radiation (energy) by the matter. Spectroscopy are of two types, absorption spectroscopy and emission spectroscopy. The study of electromagnetic radiation absorbed by the sample, in the form of spectra is called absorption spectroscopy (UV-visible, 1R, NMR, microwave and Radiowave spectroscopy). The study of electromagnetic radiation emitted by the sample, in the form of spectra is called emission spectroscopy (Flame photometry and fluorimetry). Spectroscopy is useful for the study of atomic and molecular structure and used in the analysis of a wide range of samples. Atomic spectroscopy is the study of interaction of electromagnetic radiation with atoms, changes in energy takes place at atomic level (e.g. atomic absorption spectroscopy and flame photometry). Molecular spectroscopy is the study of interaction of electromagnetic radiation with molecules, changes in energy takes place at molecular level (e.g. ultraviolet and infrared spectroscopy) (Chatwal and Anand, 2002).

In UV-visible spectroscopy, the amount of light absorbed at each wavelength of UV and visible region of electromagnetic spectrum is measured. This absorption spectroscopy uses electromagnetic radiations between 200 nm to 800 nm and is divided into the ultraviolet (UV, 200-400 nm) and visible (VIS, 400-800 nm) regions. The principle of UV-Visible spectroscopy is based on the absorption of ultraviolet light or visible light by sample or chemical substance which results in the production of different spectra. When a molecule absorbs UV radiation, the electron present in that molecule undergo excitation, this causes transition of electron within a molecule from a lower level to a higher electronic energy level and the ultraviolet emission spectra arise from the reverse type of transition. Applications of UV spectroscopy are detection of functional groups, detection of conjugation, detection of geometrical isomers and detection of impurities (Chatwal and Anand, 2002).

EXPERIMENTAL METHODS:-

Determination of percentage yield

The percentage yield of the extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

Phytochemical screening

Phytochcmical tests arc analytical procedures used to identify and quantify the presence of phytochemicals in plants and plant-based materials. Phytochemicals are naturally occurring compounds found in plants that arc known to have potential health benefits and biological activities.

1. **Detection of alkaloids:** Extract were dissolved individually in dilute Hydrochloric acid and filtered

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

2. **Detection of carbohydrates:** Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: Filtrates was hydrolysed with

dil. HC1, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extract was hydrolysed with dil. HC1, and then subjected to test for glycosides.

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of cone, nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Total alkaloid content estimation

Total alkaloid content estimation is an important analytical process in the study of plants, pharmaceuticals, and natural products. Alkaloids, a diverse group of naturally occurring compounds containing basic nitrogen atoms, are known for their significant pharmacological properties. The estimation of total alkaloid content provides insight into the medicinal potential of plant materials and aids in standardizing herbal products. This process typically involves the extraction of alkaloids using solvents, followed by quantification through various techniques such as gravimetry, titrimetry, or advanced chromatographic methods. Accurate determination of alkaloid content is essential for ensuring the efficacy, safety, and quality of herbal medicines and pharmaceutical formulations.

The estimation of total alkaloid content using bromocresol green (BCG) solution is a well-established method that leverages the interaction between alkaloids and the BCG dye. Bromocresol green is an acid-base

indicator that forms a complex with alkaloids, leading to a measurable color change. In this method, the alkaloids are first extracted from the plant material using an appropriate solvent, such as chloroform. The extract is then treated with BCG solution, resulting in the formation of a yellow-colored complex in an acidic medium. The intensity of the color, which correlates with the concentration of alkaloids, is measured using a spectrophotometer at a specific wavelength, typically around 470 nm. This technique is valued for its simplicity, sensitivity, and ability to provide a quantitative estimation of the total alkaloid content in various plant extracts and pharmaceutical preparations.

The plant extracts (Img) was dissolved in methanol, added 1ml of 2 N HC1 and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 pg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/1 OOmg of extract (Shamsa*et al.,* 2008).

Total phenolic content estimation

Total phenol content is an important analytical parameter used to assess the presence and concentration of phenolic compounds in various substances such as plant extracts, foods, beverages, and environmental samples. Phenolic compounds are a diverse group of secondary metabolites found abundantly in nature, particularly in fruits, vegetables, and medicinal plants. Phenolic compounds are characterized by the presence of one or more hydroxyl groups attached to an aromatic ring. They exhibit a wide range of biological activities and are known for their antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. These compounds play a crucial role in plant defense mechanisms against pathogens, pests, and environmental stresses. The determination of total phenol content is often performed using spectrophotometric methods based on the ability of phenolic compounds to react with specific reagents. The most commonly employed method is the Folin-Ciocalteu assay, which relies on the reduction of the Folin-Ciocalteu reagent by phenolic compounds under alkaline conditions, leading to the formation of a blue-colored complex. The intensity of the color is directly proportional to the total phenol content and can be quantified using a spectrophotometer.

The total phenol content is expressed as milligrams of gallic acid equivalents (GAE) per gram or milliliter of the sample. Gallic acid is often used as a standard reference compound because of its widespread occurrence in natural sources and its similar chemical reactivity to other phenolic compounds.

RESULTS AND DISCUSSION:-

Results of % yield

The variation in % yield among different solvent extracts underscores the importance of solvent

selection in extraction processes, as it can significantly influence the composition and efficacy of the resulting extracts for various applications in pharmaceuticals, nutraceuticals and natural product research.

Table - 1: % Yield of extract of *Embeliaribes*

S. No.	Extract	% Yield (W/W)
1.	Ethanolic extract	9.6%

The extraction yield of *Embeliaribes* using ethanol as a solvent was determined to be 9.6% (w/w), as shown in Table -1. This yield reflects the efficiency of the ethanolic extraction process in isolating the bioactive compounds from the plant material. The 9.6% yield of the ethanolic extract of *Embeliaribes* demonstrates the solvent's effectiveness in extracting bioactive compounds from the plant.

Result of phytochemical screening of extract of *Embeliaribes*

Table -2: Result of phytochemical screening of extract of *Embelia ribes*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids** Wagner's Test:	+ve
2.	**Glycosides** Legal's Test:	+ve
3.	**Flavonoids** Alkaline Reagent Test: Lead acetate Test:	-ve +ve
4.	**Diterpenes** Copper acetate Test:	-VC
5.	**Phenol** Ferric Chloride Test:	+VC
6.	**Proteins** Xanthoproteic Test:	+ve
7.	**Carbohydrate** Fehling's Test:	-VC
8.	**Saponins** Froth Test:	-VC
9.	**Tannins** Gelatin test:	+ve

+ve= Positive; -ve= Negative

The phytochemical screening of the ethanolic extract of *Embeliaribes* revealed the presence of several bioactive constituents. The extract tested positive for alkaloids, as indicated by Wagner's test, which suggests the potential medicinal properties of this plant due to the pharmacological activities commonly associated with alkaloids. Glycosides were also detected through Legal's test, highlighting their possible role in therapeutic applications, such as cardiac health. The presence of flavonoids was confirmed by the lead acetate test, though the alkaline reagent test yielded a negative result, pointing to the selective presence of certain types of flavonoids. Diterpenes were not detected in the extract, as shown by the negative copper acetate test, suggesting their absence or minimal presence in this plant. The presence of phenols was confirmed by the ferric chloride test, indicating the potential antioxidant properties of the extract. Proteins were found to be present as well, as evidenced by the positive xanthoproteic test, suggesting the nutritional or enzymatic relevance of the extract. Interestingly, carbohydrates and saponins were absent, as indicated by the negative Fehling's and froth tests, respectively. Lastly, the presence of tannins was confirmed by the gelatin test, suggesting that the extract may possess astringent and antimicrobial properties.

Total alkaloid content estimation (TFC).

Total alkaloid content was calculated as atropine equivalent (mg/lOOmg) using the equation based on the calibration curve: **y = 0.006x - 0.003, R^2=0.999,** where X is the atropine equivalent (AE) and Y is the absorbance.

Table - 3: Preparation of Calibration curve of Atropine

S. No.	Concentration (pg/ml)	Mean absorbance
1	40	0.245±0.001
2	60	0.377±0.003
3	80	0.504±0.005
4	100	0.622±0.002
5	120	0.763±0.004

(n=3; mean ± SD)

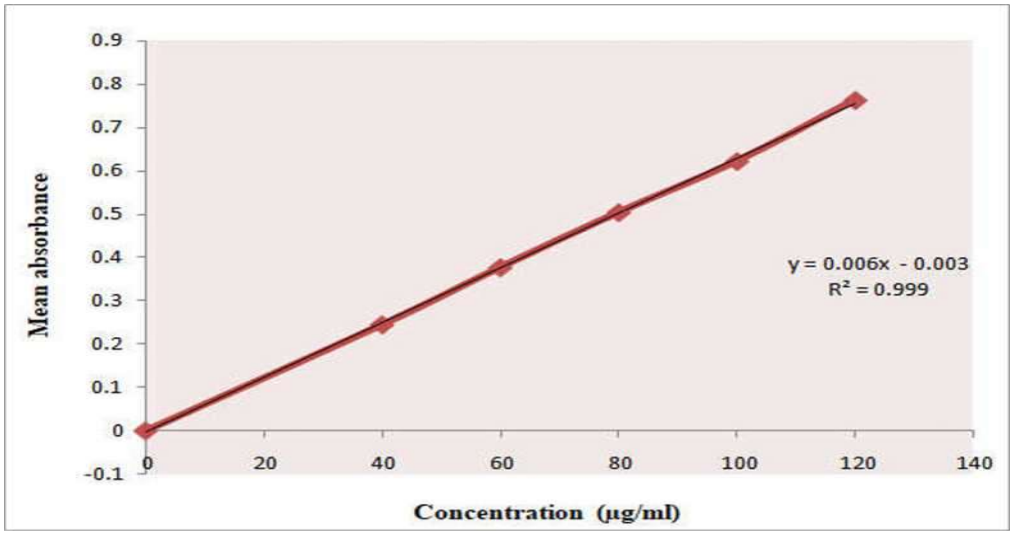

Figure 1: Graph of calibration curve of Atropine

Total phenolic content estimation (TPC)

The content of total phenolic compounds (TPC) content was expressed as mg/IOOmg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: y = 0.02x + 0.008, R^2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Table- 4: Preparation of calibration curve of Gallic acid

S. No.	Concentration (pg/ml)	Mean absorbance
1	10	0.217+0.002
2	20	0.402+0.003
3	30	0.615+0.004
4	40	0.821±0.002
5	50	0.993+0.001

(n=3; mean±SD)

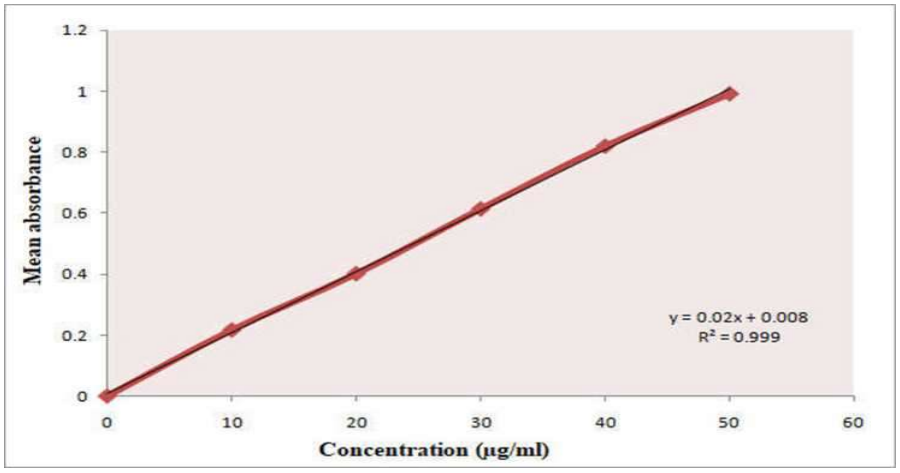

Figure 2: Graph of estimation of total phenolic content

Table - 5: Estimation of total alkaloid and phenol content of *Embeliaribes*

S. No.	Extract	Total alkaloid content (mg/100 mg of dried extract)	Total phenol content (mg/100 mg of dried extract)
1.	Ethanol	0.836	0.474

The estimation of total alkaloid and phenol content in the ethanolic extract of *Embeliaribes*reveals the presence of both bioactive compounds in measurable quantities. The total alkaloid content was found to be 0.836 mg per 100 mg of dried extract, indicating a significant presence of alkaloids, which are known for their pharmacological effects such as

antimicrobial, anti-inflammatory, and analgesic properties. The total phenol content was determined to be 0.474 mg per 100 mg of dried extract, suggesting that phenolic compounds, which are often associated with antioxidant activity, are also present in the extract. These findings suggest that the ethanolic extract of *Embeliaribes* possesses a considerable amount of both alkaloids and phenols, which could contribute to its potential therapeutic applications. Further studies could explore the specific alkaloids and phenols present and their respective biological activities.

SUMMARY AND CONCLUSION:-

The study successfully extracted and characterized the bioactive compounds present in *Embeliaribes.* The ethanol extraction yielded 9.6% (w/w) of extract, indicating a substantial recovery of phytochemicals from the plant material. Phytochemical screening revealed a diverse profile of constituents. Alkaloids and glycosides were present, as confirmed by Wagner's and Legal's tests, respectively. Flavonoids were detected using the Lead acetate test but not the Alkaline reagent test, suggesting their selective presence. Phenolic compounds were identified through Ferric Chloride testing, and proteins were confirmed by Xanthoproteic testing. Tannins were also present, as evidenced by the Gelatin test, while diterpenes, carbohydrates, and saponins were absent.

Quantitative analyses highlighted the extract's content of active compounds. The total alkaloid content was 0.836 mg/100 mg of dried extract, measured against a calibration curve of atropine, and the total phenolic content was 0.474 mg/100 mg, based on a calibration curve with gallic acid. The HPTLC analysis provided a detailed calibration curve for Embelin, showing a strong linear correlation between concentration and area, which underscores the precision of the method. The percentage of Embelin in the hydroalcoholic extract was determined to be 0.65%.

In conclusion, the ethanol extract of *Embeliaribes* exhibits a rich array of bioactive compounds, with notable levels of alkaloids, glycosides, phenols, and proteins. The HPTLC analysis confirms the presence of Embelin, demonstrating the method's accuracy in quantifying this key component. These findings emphasize the extract's potential as a source of valuable therapeutic agents and provide a solid foundation for further pharmacological research and development.

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