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Original Article

Pharmacognostical and Preliminary Phytochemical Investigations on fruit of *Vaccinium macrocarpon* aiton

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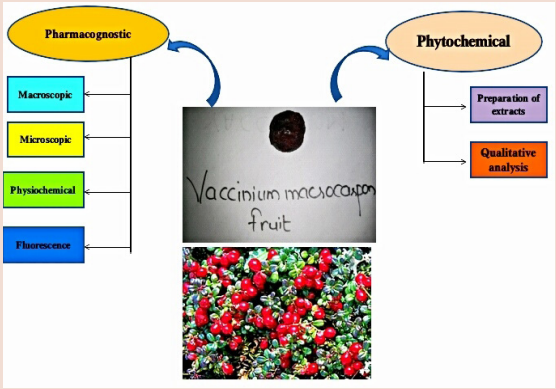
ABSTRACT

Background: *Vaccinium* species are hostile nutraceutical fruit in India as well as all over the world. In recent years, *Vaccinium macrocarpon* Aiton is used as a functional food for treating various diseases without authentication. **Objective:** The current work was investigated to perform the morphoanatomical and physicochemical of *Vaccinium macrocarpon* Aiton fruit. **Method:** Pharmacognostic studies were carried out for different parameters include organoleptic, macroscopic, microscopic, fluorescence and physicochemical analysis. **Results:** The fruit was shining burgundy purple in colour having smooth lustrous surface, globular to ellipsoidal in shape with 10-15 mm in length and diameter was 9 mm. The main microscopic characteristic of fruit showed ovules, compact angular parenchyma cells, developed sclerenchymatous outer sheath, central xylem and phloem strands. Fruit powder showed oil bodies, spherical parenchyma cells in large thick masses and walls of the epicarp demonstrated cellulose content. Further, physicochemical examination of fruit powder showed loss on drying, total ash, insoluble ash as 9.23, 7.8, and 9.16% w/w respectively. The water and alcohol soluble extractives values of the fruit were 24.74 and 76.88% respectively. Anthocyanins and flavonids were also confirmed by phytochemical screening. **Conclusion:** A variety of pharmacognostic features was found in fruitful way which may help in identification and standardization of *Vaccinium macrocarpon* Aiton fruit in a crude form.

Key words: Fruit, Morphoanataomical, Microscopy, Physicochemical analysis, *Vaccinium macrocarpon* aiton.

SUMMARY

- *Vaccinium macrocarpon* Aiton is a Nutraceutical food having potential pharmacological properties for the treatment of various disorders. It is also known as cranberry fruit.
- A fruit is having different types of flavonoids and isoflavonids. The main constituents are anthocyanins and quercetin category.
- Our study of this fruits shows phytochemical and pharmacognostical results and it is having great importance in its field.



PICTORIAL ABSTRACT

Abbreviations used: TBA: Tertiary butyl alcohol, µm: micro meter, gm: gram, h: hour, mm: milli meter, CVS: Carpellary vascular strand, Ep: Epidermis, MC: Mesocarp, Ph: Pholem, Se: Septa, SE: Sub epidermal layer, X: Xylem, OB: Oil body, PC: Parenchyma cells, Pc: Pericarp, CC: Carpellary chamber, VS: Vascular strand.

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INTRODUCTION

Ericaceae or heath family is the largest family in Ericales containing 128 genera and 4,000 species all over the world. Still many new species are being discovered. Numerous member of ericaceae are important in horticulture (e.g., rhododendrons, azaleas) or in small fruit crops (cranberries, blueberries).[1] *Vaccinium* (Ericaceae) is a genus of around 450 species of deciduous, evergreen dwarf, prostrate, or erect shrubs, vines, and trees includes a wide range of edible berries such as cranberry, blueberries, tackleberries, hurtleberries, huckleberries, farkle berries, sparkleberries, deer berries, and southern gooseberries.[2] There are many subgenera and several sections such as subgenus *Oxycoccus* (Section *Oxycoccus*), subgenus *Vaccinium* (Section *Batodendron, Brachyceratium, Bracteata, Ciliata, Cinctosandra, Conchophyllum, Cyanococcus, Eococcus, Epigynium, Hemimyrtillus, Myrtillus, Neurodesia, Oarianthe, Oreades, Pachyanthum, Polycodium, Pyxothamnus, Vaccinium, Vitis-idaea*).

Berry fruit belongs to *vaccinium* species are used as a medicinal food across the world. Very few species are available in India like *Vaccinium neilgherrense* Wt, *Vaccinium myrtillus* and *Vaccinium leschenaultia* wt. An American cranberry *Vaccinium macrocarpon* Aiton which is generally known as "large cranberry" having diploid in nature present in various part of the geographical regions like Northeastern USA, British Columbia, Europe, North America, Central America, Central and South East Africa, Madagascar, Japan and Asia. Cranberries have their own important role as a traditional health improve agent and produced in the form of fruit, concentrated juices, jams, chocolates, candy's, sauces and spray-dried powders.[3]

Now days, a large cranberry (*V. macrocarpon*) is one of the considerable nutraceutical source in functional foods industry. It is best known for the treatment and prevention of urinary tract infections (UTIs) from last many years. Cranberries and cranberry constituents have also been shown to possess antibacterial,[4-5] anti-viral,[6] anti-mutagenic,[7] anti-carcinogenic,[8] anti-tumorigenic,[9] anti-angiogenic[10] and antioxidant activities.[11] Several common chemical constituents are present among all *vaccinium* species which possess pharmacological properties. Cranberries contain a high amount of phenolic acid such as benzoic acid, hy-

droxycinnamic acid p-coumaric, sinapic, caffeic and ferulic acid. The predominant flavonoids are flavonols, flavan-3-ols (particularly proanthocyanidins).[12] The six major anthocyanins are peonidin-3-galactoside, cyanidin-3-galactoside, cyaniding-3-arabinoside, peonidin-3-arabinoside, peonidin-3-glucoside and cyaniding-3-glucoside.[13-15] A review of literature revealed that no pharmacognostic standards have been recorded for this nutraceutical drug. Hence the present investigation was undertaken to evaluate various pharmacognostical standards like macroscopic and microscopy of fruits and its powder; physiochemical values and preliminary analysis of *Vaccinium macrocarpon* fruits so that authentic plant material could be explored for its therapeutic claim.

MATERIAL AND METHODS

Procurement of plant materials

Vaccinium macrocarpon, fruits were purchased from Shaanxi Nigbo Extracts Company Ltd, China, during December 2011. The fruits were authenticated with vide batch number CB091012 from the company. The fresh fruits material was cleaned with distilled water to remove debris and dried at 35-40°C for 10 days and, pulverized in the electric grinder and the powder was passed through sieve no. 60 and stored in airtight container for further extraction.

Chemicals and instruments

All the chemicals used in the study were of analytical grade and were obtained from Rankem limited India and Nice chemicals. Compound microscope, glass slides, cover slips, watch glass and other common glass ware were the basic apparatus and instruments used for the study. Microphotographs were taken using a motic images microscope.

Anatomical evaluation

Anatomy provides one to discover convincing diagnostic characters for a specific plant species (of crucial importance in quality control), and it also allows one to observe the distribution of compounds in the plant matrix.

Macroscopic study

Various organoleptic features and morphological characters of *Vaccinium macrocarpon* fruit like color, shape, size, odour, taste and fracture were evaluated.

Microscopic study

For microscopic evaluation, fruits were cut and fixed in chemical (Formalin 5 ml + acetic acid 5 ml + 70% ethyl alcohol-90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary–butyl alcohol (TBA) as per the procedure of Sass, 1940.[16] Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was by customary procedure.[17] The characteristic structures and cell components were observed and their photographs were taken using photomicrography.

Physiochemical analysis

In this study, dried fruit material was used for quantitative determination of physiochemical values like loss on drying, total ash, acid insoluble ash, water soluble ash, sulphated ash values and extractive values were determined as per reported method.[18]

Fluorescence analysis

Fluorescence study of fruit powder was performed as per reported standard procedure.[19] A small quantity of the fruit powder was placed on a grease free clean microscopic slide and 1-2 drops of the freshly prepared reagent solution were added, mixed by gently tilting the slide and waited for 1-2 min. then the slide was placed inside the UV chamber and observed in visible light, short (254 nm) and long (365 nm) ultraviolet radiations. The colours observed by application of different reagents in different radiations were recorded.

Preparation of extracts

Fruit powder material (10 gm) was macerated by exhaustive method for preparation of three different extracts using acetone water (70:30), Ethanol and water (40: 60) with inorganic salt (2.5 gm) and methanol, water and ethyl acetate (80:19.5:0.5), Each combination were used for 72 h then 48 h and lastly 24 h. the last trace solvents was removed by vacuum drying method. Yields were calculated on the basis of percentage w/w. The extracts were stored below 4°C until further used. The extracts were concentrated by performing the qualitative chemical tests to determine various chemical constituents and investigated fluorescence analysis.[20]

RESULTS

Macroscopic study of fruit

Morphological evaluation (Figure 1) of the fruit showed shines burgundy purple in colour. The shape was globular to ellipsoidal and the size about 10-15 mm in length and having diameter 9 mm. This fruits possess smooth lustrous surface showed characteristics in odor and sweet taste.

Figure 1: Fruits of *Vaccinium macrocarpon*

Microscopic study of fruit

Transverse section of fruit demonstrated spherical, fleshy berry with 14 mm thickness. It is tetra carpellary with many ovules on axile placentation. The fruit consists of thin layers of epidermis with prominent cuticle and a few layers of compact angular parenchyma cells. This portion represents the epicarp of fruit. The remaining portion of the fruit is thick a parenchymatous which represents the mesocarp of the fruit. The mesocarp includes several layers of wide parenchyma cells with wavy thin cell walls. There are small circular vascular strands distributed in the mesophyll tissue (Figure 2a and 2b). The vascular strands of the pericarp are circular with well developed sclerenchymatous outer sheath and central xylem and phloem strands (Figure 2c). The fruit is divided into 4 carpels by thick wavy septa (Figure 3a and 3b). The septa are thick and consist of epidermal layers and inner parenchymatous tissue. In the centre of the union of the 4 septa there are four circular vascular strands. These vascular strands are called carpellary vascular strands. In the pericarp, the

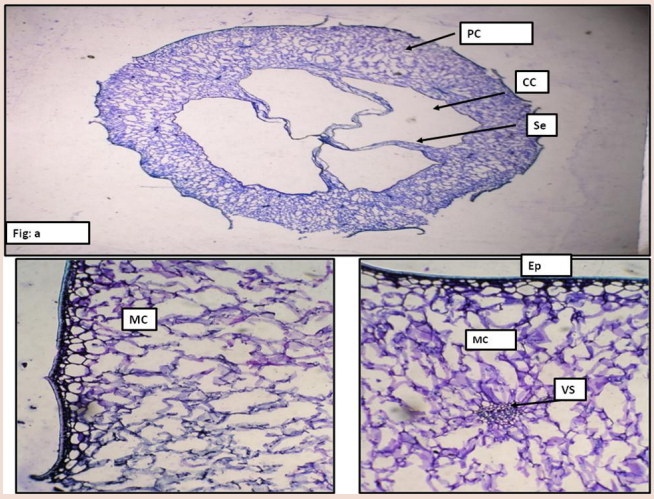

Figure 2: a) Transverse section of fruit entire view b) Epicarp and outer carp mesocarp of the fruit c) Mesocarp with circular vascular bundle
(CC: Carpellary chamber, EP: Epidermis, MC: Mesocarp, Se: Septa, Pc: Pericarp, VS: Vascular strand)

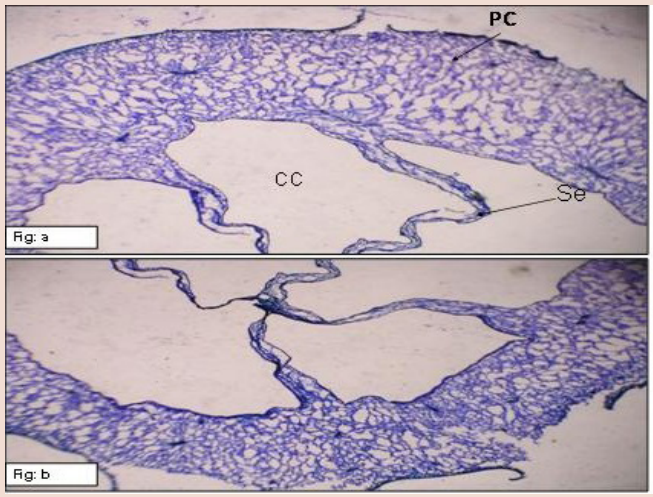

Figure 3: a) and b) Transverse section of fruit showing two halves of the fruit (CC: Carpellary chamber, Pc: Pericarp, Se: Septa)

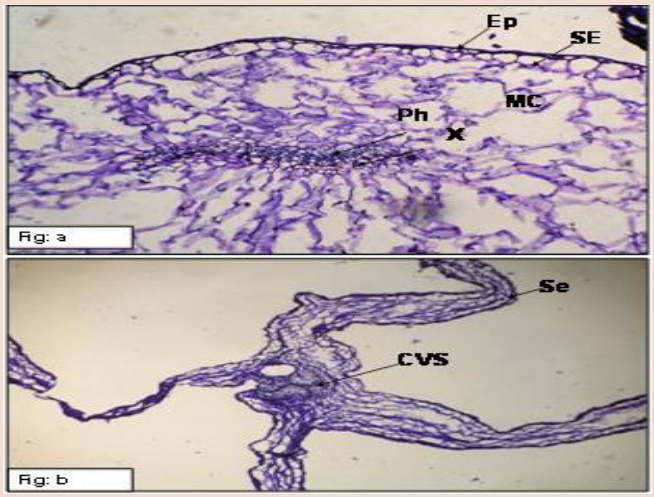

Figure 4: a) Transverse section of pericarp of the fruit showing a flat vascular strand b) Central part of the septa showing of circularly carpellary vascular strands
(CVS: Carpellary vascular strand, Ep: Epidermis, MC: Mesocarp, Ph: Pholem, Se: Septa, SE: sub epidermal layer, X: xylem)

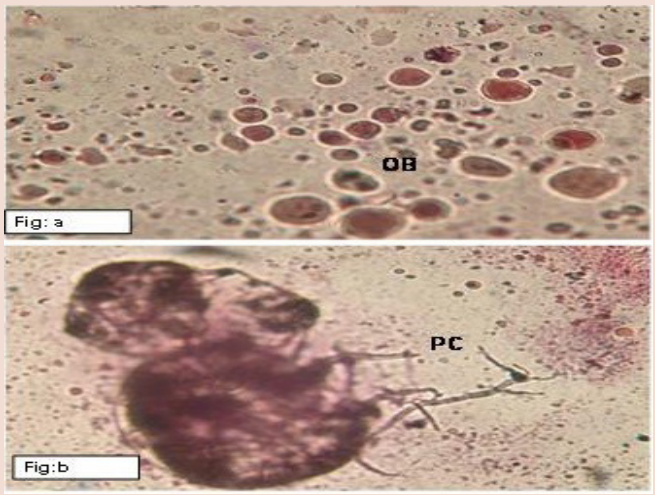

Figure 5: a) Fruit powder showing droplets of oil bodies b) Parenchyma cells of the pericarp showing cell inclusions
(OB: Oil body, PC: Parenchyma cells)

vascular strands may be flat (Figure 4a) or circular (Figure 4b). The flat vascular strands have a transverse row of xylem elements with phloem located on the outer part of the xylem strand (Figure 4a). The pericarp of the fruit is 350 μm thick.

Powder microscopy

The powder of the fruit showed oil bodies which was variable in shape and size. They were found floating appeared brown in color and stained with neutral red (Figure 5a). With addition to this, it showed spherical parenchyma cells in large thick masses (Figure 5a) or small group of 2 or 3 cells composed of oil bodies which stain dark brown (Figure 5b). On the surface, small pieces of epicarp were seen in surface view. These pieces consist of polyhedral thick walled parenchyma cells (Figure 6a and 6b). The walls of the epicarp have cellulose content and the cells have wide empty lumen.

Fluorescence analysis

The Fluorescence characteristics of the fruit powder with different chemical reagents and fruit extracts are summarized in Table 1 and Table 2.

Physicochemical Parameters

In this study, various physicochemical parameters like loss on drying, total ash, acid insoluble ash, water soluble ash, sulphated ash and extractives values were determined in triplicate as mentioned in Table 3. The total yield and morphological characteristics of extract was shown in Table 4.

The qualitative results (Table 5) of all three extracts of *Vaccinium macrocarpon* fruit showed the presence of carbohydrates, flavonoids and anthocyanins, while these extracts could not show any positive indication for proteins, amino acids and alkaloids. The presence of flavonoids and

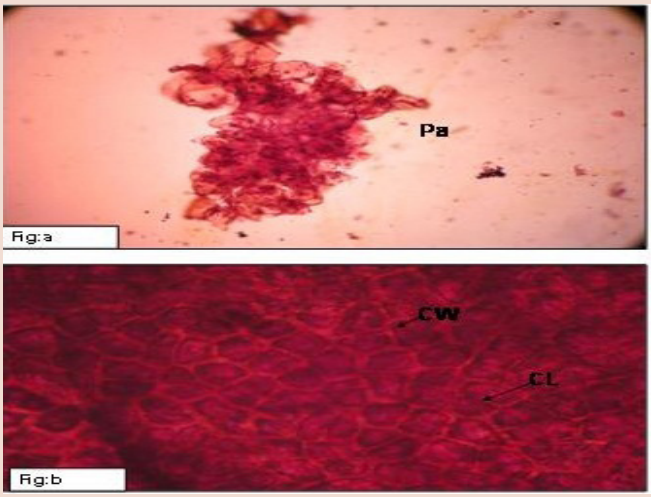

Figure 6: a) A thick mass of mesocarp parenchyma cells b) Surface view of the epicarp (epidermis of the fruit
(Cw: Cell wall, Pa: Parenchyma)

Table 1: Fluorescence analysis of *VacciniuWm macrocarpon* fruit powder

Treatment	Under UV light		Visible light
	Short wavelength (254 nm)	Long wavelength (365 nm)	
Powder as such	Dark Brown	Brownish Black	Blackish Brown
Powder + 5% NaOH	Cherry Brown	Brownish Black	Brown
Powder + 5% KOH	Cherry Brown	Dark Black	Cherry Brown
Powder + 5% $FeCl_3$	Cherry Brown	Brownish Black	Cherry Brown
Powder + conc H_2SO_4	Dark Black	Reddish Black	Brownish Black
Powder + conc HCl	Greenish Brown	Blackish Brown	Brownish Black
Powder + conc HNO_3	Greenish	Reddish Black	Yellowish Brown

Table 2: Fluorescence nature of various extracts of *Vaccinium macrocarpon* fruit powder

Treatment	Under UV light		Visible light
	Short wavelength (254 nm)	Long wavelength (365 nm)	
Acetone–Water (70:30)	Yellowish Black	Dull Brown	Yellowish Black
Ethanol + Inorganic salt + Water (40:60)	Yellowish Black	Black	Brownish Black
Methanol + Ethyl Acetate + Water (80:0.5:19.5)	Brown	Blackish Brown	Brownish Black

Table 3: Physicochemical analysis of *Vaccinium macrocarpon* fruit

Parameters	Value obtained on dry weight basis (% w/w)
Loss on Drying	9.236 ± 33.09
Water soluble extractives	76.88 ± 5.19
Alcohol soluble extractives	24.74 ± 4.22
Total ash	7.8 ± 12.07
Acid Insoluble ash	9.1666 ± 11.33
Foreign matter	Nil

anthocyanins was also confirmed by TLC method which showed pinkish violet colour having R_f values, which was found in the range of 0.15-0.95 (data not shown).

DISCUSSION

Ethno medicinally, the fruit of this plant has been use by many people in the treatment of various diseases especially in cancer and ulcers without knowing any pharmacognostical authentication. The pharmacognostical standardization of a crude drug is an important tool for identifying any morphological and physicochemical characters and it is helpful to add in different pharmacopeias. Two methodologies has been used for evaluating pharmacognostic parameters one is microscopic and another is macroscopic. Till now, no pharmacognostic work has been done on this medicinally potent plant fruit. The present work was undertaken to lay down the standards which could be useful for establishing authenticity. The macroscopic characters of fruit served as diagnostic parameters which possess fractured surfaces of fresh and dried fruit, typically tongue sensitizing aromatic taste and odour. Ash and extractive values are also

Table 4: Morphological characteristics and percentage yield of *Vaccinium macrocarpon* fruit extract

Extract	% dry wt (mg)	Colour	Odour	Consistency	% Yield
Acetone–Water (70:30)	154.15	Brownish Black	Odourless	Semi-solid viscous	15.41
Ethanol+ Inorganic salt + Water (40:60)	174.4	Brownish Black	Odourless	Semi-solid viscous	17.44
Methanol + Ethyl Acetate + Water (80:0.5:19.5)	70.45	Brownish Black	Odourless	Semi-solid viscous	7.045

Table 5: Phytochemical test of *vaccinium macrocarpon* fruit

Constituents	Phytochemical test	Extracts		
		Acetone-water (70:30)	Ethanol+ Inorganic salt + Water (40:60)	Methanol + Ethyl Acetate + Water (80:0.5:19.5)
Carbohydrates	a. Molisch test	+ +	+ +	+ +
	b. Benedict test	+ +	+ +	+ +
	c. Fehling test	+ +	+ +	+ +
Proteins	a. Biuret test	– –	– –	– –
	b. Millon's test	– –	– –	– –
Amino-Acids	a. Ninhydrin test	– –	– –	– –
		– –	– –	– –
Flavonoids	a. Sulphuric acid	+ +	+ +	+ +
	b. Lead acetate	+ +	+ +	+ +
	c. Ferric Chloride			
Alkaloids	a. Dragendroff	– –	– –	– –
	b. Mayer test	– –	– –	– –
	c. Wagner test	– –	– –	– –
Anthocyanins	a. Sodium Hydroxide	+ +	+ +	+ +
	b.$KMnO_4$ Discoloration			

reliable aid for detecting adulteration. Ash values are use to give an idea about inorganic composition and other impurities present in drug and extractive values shows the chemical constituents present in the crude drug and also helpful in estimation of specific constituents which are soluble in particular solvents. The fluorescent analysis under day light and UV light by treatment with different chemical reagents showed different color which suggested the presence of active chemical constituents. As per phytochemical screening, the fruit of *Vaccinium macrocarpon* showed mainly anthocyanins and flavonoids which was also reported in previous study.[21-22]

As per our results, water soluble extractive value indicated the presence of sugar and inorganic compounds where as alcohol soluble extractive value indicated the presence of polar constituents such as glycosides, anthocyanins and flavonoids similar results were also reported by previous researcher.[23]

CONCLUSION

From these parameters, the results are being useful for setting up some diagnostic indices for identification and preparation of monograph according to pharmacopeia. Other related species can also be compared from these parameters and thus would helpful in opening up of new avenues in the use of natural products for therapeutic purposes. Some of the manufacturers can also utilize these data for preparation of formulation according to their needs. Many therapeutics like anti diabetic, anti obesity and anti hypertensive activity are still lacking of this plant, for future pharmacological study, these data can be useful for collection and identification of this plant.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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