

PHYTOCHEMICAL ANALYSIS OF FRUIT EXTRACT OF *MYRSINE AFRICANA*

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ABSTRACT

The present paper deals with the phytochemical screening of therapeutic importance from *Myrsine africana*, an important medicinal plant. This study involves the preliminary screening and quantitative determination of secondary metabolites from the fruits of *M.africana*. The phytochemical analysis revealed the presence of saponins, tannins, flavonoids, amino acids, steroids and reducing sugar. The amount of the saponins and tannins in methanolic extracts are reported (17.5% and 4% respectively). The generated data has provided the basis for its wide use as the therapeutant both in the traditional and folk medicines.

Keywords: *Myrsine Africana*, Fruits, Phytochemical analysis.

INTRODUCTION

In recent times, there have been increased waves of interest in the field of Research in Natural Products Chemistry¹. Plants have been used as treatments for thousands of years, based on experience and folk remedies and continue to draw wide attention for their role in the treatment of mild and chronic diseases². The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals). These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others. Nearly 50% of drugs used in medicine are of plant origin, and only a small fraction of plants with medicinal activity has been assayed. There is therefore much current research devoted to the phytochemical investigation of higher plants which have ethnobotanical information associated with them³.

Plants have an almost limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as the molecules of plant defense against predation by microorganisms, insects, and herbivores. Further, some of which may involve in plant odour (terpenoids), pigmentation (tannins and quinines), and flavour (capsacin). However, several of these molecules possess medicinal properties⁴.

Myrsinaceae, or the Myrsine family, is a rather large family from the order Ericales. It consists of 35 genera and about 1000 species. They are mostly mesophytic trees and shrubs; a few are lianas or sub-herbaceous⁵. It is a large family of woody plants with leaves that are gland-dotted and the fruits are a berry. The small genus *Myrsine* has about ten species that occur from Africa to China. Two species, *Myrsine africana* and *M. pillansii* are indigenous to South Africa⁶.

Myrsine africana is an important medicinal plant, from this family, used in Ayurveda, Unani, Siddha, and in folk medicine for treating several ailments⁷. Pharmacologically the aerial parts of plant are found to possess anti-tumor⁸, purgative⁹, anti-fertility¹⁰, anthelmintic¹¹, phytotoxic¹², haemagglutination activity¹² and antimicrobial¹³.

In the present study, we have concentrated on the preliminary screening and quantitative determination of secondary metabolites from the fruits of *Myrsine africana*.

MATERIALS AND METHODS

Collection and identification of plant

The fruits of the plant were collected throughout the month of September, 2010 from three different locations of Himachal Pradesh (Solan, Shimla, Sirmour) in India. The plant species were identified and authenticated by Dr. R. Raina, qualified taxonomist from the Department of Forest Products, Dr. Y.S. Parmar University of

Horticulture and Forestry, Nauni, Solan (H.P.). Voucher specimens were deposited with the Herbarium at Nauni and are entered in the UHF-Herbarium Field book no. 5585 dated 14.09.2010.

Preparation of extracts

Aqueous extract

The aqueous extract of fruits of *Myrsine africana* was prepared by the method of Decoction:

Procedure: The sample (fruits of *Myrsine africana* 30gm) was weighed and soaked in 400ml of distilled water in two different beakers. It was then exposed to a continuous slow heat followed by stirring at an interval of 3-5min and was brought to a boil until the total volume reached 1/3 of its original size. The decoction was then taken off the heat and strained through a fine filter paper. The filtrate was then concentrated over the hot water bath to get a semi-solid.

Methanolic extract

The methanolic extract of fruits of *Myrsine africana* was prepared by the method of Soxhlet extraction:

Procedure: The sample (pulverised fruits of *Myrsine africana* 40gm.) was weighed and placed in the thimble made from thick filter paper, which was then loaded into the main chamber of the Soxhlet extractor. The extractor was then placed onto a flask containing the extraction solvent (methanol 500ml). The Soxhlet was then equipped with a condenser. The solvent was heated to reflux. The chamber containing the solid material was slowly filled with warm solvent to dissolve some of the desired compound. When the Soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle was allowed to repeat many times, over 36 hrs. During each cycle, a portion of the non-volatile compound dissolved in the solvent. After many cycles the desired compound was concentrated over the hot water bath to remove the solvent.

Phytochemical Screening

Chemical tests were carried out on the aqueous and methanolic fruit extracts for the qualitative determination of phytochemical constituents as described by Harborne (1973)¹⁴, Trease and Evans (1989)¹⁵ and Sofowora (1993)¹⁶.

Alkaloids

0.5 g of extract was diluted with 10 ml of acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendoff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown

precipitate (with Dragendoff's reagent) was regarded as positive for the presence of alkaloids.

Saponins (Frothing test)

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. An appearance of creamy mass of small bubbles indicated the presence of saponins.

Tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration indicating the presence of tannins.

Flavonoids (Shinoda Test)

To the test solution add few fragments of magnesium ribbon and add concentrated Hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

Steroids

Two millimeter of acetic anhydride was added to 0.5 g of ethanol extract of each sample with 2 ml H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Terpenoids (Salkowski method)

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids.

Cardiac glycosides (Keller-Killiani test)

To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Anthraquinone Glycosides (Borntrager's test)

0.5 g of the plant extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added. A pink, red or violet coloration in the ammoniacal phase indicated the presence of anthraquinone.

Amino acids (Ninhydrin test)

Amino acids and proteins when boiled with few drops of 5% solution of Ninhydrin, violet colour appears.

Carbohydrates (Molisch's test)

Treat the test solution with few drops of alcoholic α -naphthol. Add 0.2 ml of concentrated Sulphuric acid slowly through the sides of the test tube, purple to violet colour ring appears at the junction.

Reducing sugar (Fehling's Test)

A small amount of the each extract was dissolved in about 2 ml of distilled water and filtered. An equal amount of Fehling's solution 1 and 2 was added to the filtrate and the contents were boiled. Appearance of brick red precipitates confirmed the presence of reducing sugars.

Table 1: It shows preliminary qualitative phytochemical analysis of *Myrsine africana* fruits.

Phytochemical Analysis of <i>Myrsine Africana</i> Fruits			Inference	
S. no.	Phytoconstituents		Aqueous extract	Methanolic extract
1.	Alkaloids		-	-
2.	Saponins		-	-
	a) Frothing test		+	+
3.	Tannins			
	a) Lead acetate test		+	+
	b) FeCl ₃ test		+	+
4.	Glycosides			
	a) Borntrager's test		-	-
	b) Keller-killiani test		-	-
5.	Flavonoids			
	a) Shinoda test		+	+
6.	Amino acids		+	+
7.	Steroids & Terpenoids		+	+
8.	Carbohydrates			
	a) Molisch's test		+	+
	b) Fehling test (reducing sugar)		+	+

(Key: -ve sign indicate absence and +ve sign indicates presence of constituent)

Quantitative Phytochemical Screening

Determination of Saponins

20g of sample powder was dispersed in 200ml of 20% aqueous ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. The concentrate was transferred into 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.

60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous NaCl. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to constant weight and the saponin content was calculated in percentage¹⁷.

Determination of saponin content

Total amount of crude drug taken = 20 gm

Total amount of Saponin obtained = 3.5 gm

% age of Saponin = $3.5/20 \times 100 = 17.5\%$

Total Saponin present in the extract found to be 17.5%

Determination of tannins

Samples are analysed by adding 1 ml sample and 5 ml indigo carmine to the 500 ml flask and adding 200 ml water. Titrate this against the Potassium permanganate solution (N/40 or 0.005M) until the royal blue fades to a light green. Then titrate drop-wise until the lime green changes to yellow. Record this value as X ml.

A blank titration using 5 ml of indigo carmine alone in 200 ml water should also be carried out. The blank value should be 1 ml and should be recorded as Y ml.

Total Tannin (%) = (X-Y)/10 expressed as 'tannic acid' equivalents¹⁸.

Table 2: It shows total tannin content in methanolic extract of *M. africana* fruits

S. no.	Total Tannin Content		
	Initial volume (ml)	Final volume (ml)	Volume used (final vol. -initial vol.)
1.	1.0	2.4	1.4
2.	3.0	4.4	1.4
3.	5.0	6.3	1.3

Calculations of tannin content

Volume Used (X ml) = 1.4ml

Blank titration value (Y ml) = 1ml

Formula used = X-Y/10 * 100

Total Tannin (%) = 4%

Total Tannin present in the extract found to be 4%

RESULTS

Qualitative Phytochemical analysis was done by using colour forming and precipitating chemical reagents to generate preliminary data on the constituents of the plant extract. The chemical tests revealed the presence of tannins, saponins, flavonoids, steroids, amino acids, and reducing sugars in the aqueous and methanolic extracts of fruits of *Myrsine africana*. The results of preliminary qualitative phytochemical analysis are tabulated in Table 1. The quantitative estimation of saponin content (17.5%) in methanolic fruit extract of *Myrsine africana* and the determination of tannins done by Lowenthal Permanganate Titration method confirms tannin content to be (4%) in methanolic fruit extract of *Myrsine africana* as shown in Table 2.

DISCUSSION

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc.

Myrsine africana may be considered as a valuable plant in both ayurvedic and modern drug development areas of its versatile medicinal uses. The data drawn from the Phytochemical investigations on the crude aqueous and methanolic extract of authentic samples of fruits of *M. africana* suggests that tannins, saponins, flavonoids, amino acids, steroids and reducing sugar is present as active constituents in fruits of the shrub *Myrsine africana*. The present study shows the absence of alkaloids and glycosides [Table 1].

Saponins are plant-based anti-inflammatory compounds that may lower your blood cholesterol and prevent heart disease as well as some cancers. There is evidence of the presence of saponins in traditional medicine preparation^{19,20}.

Tannins have shown potential antiviral²¹ antibacterial²² and antiparasitic effects²³. Flavonoids are capable of modulating the activity of enzymes and affect the behaviour of many cell systems, suggesting that the compounds may possess significant antihepatotoxic, antiallergic, anti-inflammatory, antiosteoporotic and even antitumor activities^{24,25}. Plant sterols are best known for their ability to lower cholesterol²⁶. It is well-known that plant produce these chemicals to protect themselves but recent research demonstrate that they can also protect humans against diseases.

Conflict of interest

To our knowledge, the present study is the first one which systematically reports the phytochemicals present in methanolic and aqueous extract of fruits of *Myrsine africana*.

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