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QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL SCREENING OF THREE PLANTS STEM BARK AND LEAVES FROM SAPOTACEAE FAMILY

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ABSTRACT

In the present study, three plant Stem bark and Leaves were assessed for Phytochemical screening. Acetone, Chloroform and Aqueous extract of each plant were subjected to Qualitative and Quantitative Phytochemical screening. Acetone extracts of Stem bark positive for Alkaloid, Carbohydrate, Protein, Tannin, Terpenoid, Flavonoid, Saponin and Steroid for three plants. Chloroform extract of Stem bark positive for Alkaloid and Steroid for one plant. Aqueous extract of Stem bark positive for Alkaloid, Carbohydrate, Protein, Tannin, Saponin, Flavonoid and Steroid for three plants. Acetone extract of Leaves positive for Alkaloid, Carbohydrate, Protein, Glycoside, Saponin and Steroid for three plants. Chloroform extract of Leaves positive for Alkaloid, Carbohydrate, Terpenoid, Saponin and Flavonoid for one plant. Aqueous extract of Leaves positive for Carbohydrate, Protein, Glycoside, Tannin, Terpenoid, Saponin, Flavonoid and Steroid for three plants. The total Alkaloid, Tannin and Steroid were quantified in the Acetone extracts by standard Spectrophotometric method. Caffein was used as standard for the determination of total Alkaloid. Tannic acid used as the standard for Tannin. Manilkara zapota Stem bark shows the higher concentration Alkaloid whereas Madhuka indica stem bark shows higher concentration of Steroid. Madhuka indica leaves shows the higher concentration of Alkaloid and Tannin. The study reveals that the presence or absence of particular phytochemicals are determined by the polarity of Solvents used for extraction.

Keywords: Phytochemical Analysis, Madhuka Indica, Manilkara Zapota, Manilkara Hexandra.

INTRODUCTION

Plants are universally recognized as vital component of the world's bio-diversity and very essential resources for the planet. The art of healing has its origin in the ancient past of human civilization. The medicinal value of the plant lies in some of its chemical substances that produce a definite physiological action on human body.

Phytochemicals are primary and secondary metabolites occurring naturally in different parts of plants possess defence mechanism to protect them from various diseases. Primary metabolites are involved directly in growth and mechanism (Carbohydrate, Lipid and Protein) of plant and Secondary metabolites are considered as end products of primary metabolites and involved in metabolic activity (alkaloids, phenolics, sterols, steroids, essential oils, lignins and tannins etc.) they act as defence chemicals. Their absence does not cause bad effects in the plants.

A natural product plays an important role in the field of new drugs research and development, because of their low toxicity, easy availability and cost-effective. According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs. Manilkara zapota commonly known as Sapodilla belongs to the family Sapotaceae. It has been used in the indigenous system of medicine for the treatment of various ailments. Decoction of the bark used for diarrhea and fever. Leaf decoction used for fever, haemorrhage, wounds and healing.

Manilkara hexandra grows in natural wild condition and mainly propagated through seeds. Its usage has been reported mostly in the traditional medicinal system of India. The Leaf, stem, bark & fruit of the plant consist of various medicinal values such as astringent, refrigerant, aphrodisiac, alexipharmic, stomatic, anthelmintic. Madhuka indica commonly known as butter tree which belongs to family Sapotaceae. The main use of Madhuka indica are carminative, demulcents, emollient, laxative, astringent and tonic. The leaves are used in treatment of eczema and seeds are used to relieve pain in muscle and in joints.

Preliminary phytochemical screening of plants is the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy. Thus, the present study deals with the screening based on phytochemical test s of three plants Manilkara zapota, Manilkara hexandra, Madhuka indica stem bark and leaves for identifying their chemical constituents. All these plants possess different bioactivities which were later correlated with the presence of some specific phytoconstituents.

Medicinal plants are rich source of novel drugs that forms the ingredients in traditional system of medicine, modern medicine, pharmaceutical intermediates and lead compounds in synthetic drugs. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value. These compounds are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defence mechanism and protect from various diseases. The medicinal value of plants lies in some chemical substances (usually secondary metabolites) that produce a definite physiological action as the human body.

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MATERIAL AND METHOD

Plant materials

Fresh Stem bark and leaves of Manilkara zapota, Manilkara hexandra, Madhuka indica were collected from surrounding area of Talod Taluka, North Gujarat, India.

Preparation of the Plant parts

After the Collection and Identification of the Plant parts (Stem bark and Leaves) were washed thoroughly tap water in order to remove the dust and soil particles. Then the Plant parts were air dried under the shade to prevent Ultra-violet rays from inactivating the chemical constituents. (Das et al., 2010; Ncube et al.,2008). The individual Plant parts were later ground into powder form with the help of Mechanical grinder.

Procedure for Preparation of the crude extracts

Extraction Technique

From medicinally active part of plant tissue constituents, the separation of inactive part of plant tissue is called as extraction by using standard extraction procedure. Men strum is a selective solvent which is used to reduce the inert material and to get the curative part by treatment is the main objective of this procedure.

Solvent extraction

By using Soxhlet extraction method, crude plant extract was prepared. In a thimble 10 gm of powdered plant material was loaded and 300 ml solvents were also extracted independently. As a solvent water, Acetone and Chloroform was used. Till the solvent changed to colorless, the process of extraction sustained for 24 hours, in siphon tube of an extractor. Then in a beaker took extract. Then at 30- 40°C till all the solvent was evaporated, kept and heated this extract on hot plate. At 4°C in a refrigerator, the dried extract was stored for use in future phytochemical analysis.

Phytochemical Analysis

Chemical tests are performed on different organic and Aqueous extracts of each Plants with standard methods for various Secondary metabolites.

Qualitative Phytochemical Analysis

Alkaloid

Wagner's test: 2mg of extract was acidified with 1.5% v/v of hydrochloric acid a few drops of Wagner's reagent was added. A yellow or brown ppt indicate the presence of alkaloids.

Carbohydrates

Molisch's test: 2 mg of ethanolic extract was shaken with 10 ml of water, filtered and the filtrate was concentrated. To these 2 drops of freshly prepared 20% alcoholic solution of alpha- naphthol was added 2ml of conc. sulphuric acid was added so as to form a layer below the mixture red-violet ring appear, indicating the presence of carbohydrates which disappear on the addition of excess alkali.

Proteins: To 2 ml of each extract 1 ml of 40% sodium hydroxide and few drops of 1% copper sulphate were added; formation of violet color indicates the presence of peptide linkage molecules in the sample extract.

Glycosides: To 1 ml of each extract, 0.5 ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added, formation of brown ring at the interface indicates the presence of glycosides in the sample extract.

Tannin: To 1-2 ml of the ethanolic extract, few drops of 5% w/v Fecl3 solution was added. A green color indicates the presence of Gallo tannins, while brown color indicates the presence of pseudo tannins.

Terpenoids: Take 1 ml of each solvent and add 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish-brown precipitate indicates the presence of terpenoids in the extract.

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Saponins: To 2 ml of each extract, 6 ml of distilled water were added and shaken vigorously; formation of bubbles or persistent foam indicates the presence of saponins.
Flavonoids: 2 ml of each extract was added with few drops of 20% sodium hydroxide, formation of intense yellow color is observed. To this few drop of 70% dilute hydrochloric acid were added and yellow color was disappeared. Formation and disappearance of yellow color indicate the presence of flavonoids in the sample extract.

Steroids
Salkowski reaction: 2 mg of dry extract was shaken with chloroform to the chloroform layer sulphuric acid was added slowly by the sides of the test tube. Formation of red color indicated the presence of steroids.

Quantitative Phytochemical Analysis

Depending on the above Qualitative results the assay is carried out for Alkaloid, Tannin and Steroid.

Total Alkaloid content Determination

The Extract (1gm) was macerated with 20 ml of ethanol and 20% H2SO4 (1:1 v/v). The filtrate (1ml) was added to 5 ml of 60% H2SO4 was mixed with the mixture and allowed to stand for 3 hr. The absorbance was read at 565 nm.

Total Tannin Content Determination

The Ethanolic extract (1ml) was mixed with Folin-Ciocaltau reagent (0.5ml), followed by the addition of saturated sodium carbonate (Na2CO3) solution 1ml and distilled water (8ml). The reaction mixture was allowed to stand for 30 minutes at room temperature. The supernatant was obtained by centrifugation and absorbance was recorded at 725 nm using Ultraviolet-visible spectrophotometer. Increasing concentration of standard tannic acid were prepared and the absorbance of various tannic acid concentration was plotted for a standard graph. The Tannin content was expressed as mg tannic acid equivalent (TAE)/g of the sample.

Total Steroid Content Determination

1 ml of test extract of Steroid solution was transferred into 10 ml volumetric flask, Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2ml) were added followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5ml). The mixture was heated in a water bath maintained at 70±2°C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

RESULT AND DISCUSSION

Preliminary screening of Phytochemicals

The data shown in table 1 shows screening of three different extracts, i.e., Acetone extract, Chloroform extract and Aqueous extract of three plants viz. Manilkara zapota, Manilkara hexandra, Madhuka indica based on phytochemical tests. These tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes. The observations and interferences made in the phytochemical tests are presented as follows:

In Manilkara zapota stem bark Acetone extract Alkaloid, Carbohydrate, Tannin, Terpenoid, Flavonoid and Steroid are present and Chloroform extract Alkaloid and Steroid present and in Aqueous extract Alkaloid, Carbohydrate, Protein, Tannin, Saponin, Flavonoid and Steroid present. In Manilkara hexandra stem bark Acetone extract Alkaloid, Carbohydrate, Glycoside, Tannin, Terpenoid, Flavonoid and Steroid are present and in Chloroform extract all the phytochemicals are absent and in Aqueous extract Protein, Tannin, Saponin, Flavonoid and Steroid are present. In Madhuka indica stem bark Acetone extract Glycoside, Flavonoid and Steroid present and in Chloroform extract all the phytochemicals are absent in Aqueous extract Protein, Tannin, Saponin, Flavonoid and Steroid present. In Manilkara zapota leaves Acetone extract Alkaloid, Carbohydrate, Glycoside and Steroid present and in Chloroform extract Alkaloid, Carbohydrate, Terpenoid, Saponin and Flavonoid are present and in Aqueous extract Protein, Tannin, Saponin, Flavonoid and Steroid are present. In Manilkara hexandra leaves Acetone extract Alkaloid, Carbohydrate, Protein, Glycoside, Saponin and Steroid present and in Chloroform extract all the phytochemicals are absent and in Aqueous extract Carbohydrate, Protein, Glycoside, Saponin, Flavonoid and Steroid are present. In Madhuka indica leaves Acetone extract Alkaloid, Tannin and Saponin present and in Chloroform extract all the phytochemicals are absent and in Aqueous extract Glycoside, Tannin, Terpenoid, Saponin and Flavonoid are present.

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Table 1: Phytochemical composition of the Stem bark and leaves of three plants Acetone, Chloroform and Aqueous extract.

Plant Name	Crude Extract	Phytoconstituents								
		Alkaloid	Carbohydrate	protein	Glycoside	Tannin	Terpenoid	Saponin	Flavonoid	Steroid
Manilkara zapota stem bark	AE	+	+	-	-	+	+	-	+	+
	CE	+	-	-	-	-	-	-	-	+
	WE	+	+	+	-	+	-	+	+	+
Manilkara hexandra stem bark	AE	+	+	-	+	+	+	-	+	+
	CE	-	-	-	-	-	-	-	-	-
	WE	-	-	+	-	+	-	+	+	+
Madhuka indica stem bark	AE	-	-	-	+	-	-	-	+	+
	CE	-	-	-	-	-	-	-	-	-
	WE	-	-	+	-	+	-	+	+	-
Manilkara zapota Leaves	AE	+	+	-	+	-	-	-	-	+
	CE	+	+	-	-	-	+	+	+	-
	WE	-	-	+	-	+	-	+	+	+
Manilkara hexandra Leaves	AE	+	+	+	+	-	-	+	-	-
	CE	-	-	-	-	-	-	-	-	-
	WE	-	+	+	+	-	-	+	+	+
Madhuka indica Leaves	AE	+	-	-	-	+	-	+	-	-
	CE	-	-	-	-	-	-	-	-	-
	WE	-	-	-	+	+	+	+	+	-

+ = positive, - = negative, AE = Acetone extract, CE = Chloroform extract, WE = Water extract

Quantitative analysis

Table 2: Quantitative analysis for total Alkaloid, Tannin and Steroid.

Plants	Phytoconstituents		
	Total Alkaloid	Total Tannin	Total Steroid
Manilkara zapota Stem bark	2823	594	916
Manilkara hexandra Stem bark	2673	616	944
Madhuka indica Stem bark	-	-	1038
Manilkara zapota Leaves	1111	-	-
Manilkara hexandra Leaves	2145	-	-
Madhuka indica Leaves		296	-

The results of Quantitative estimation of the chemical constituents like total Alkaloid, Tannin and Steroid of Acetone extract by spectrophotometric method is summarized in table 2.

Among all the three plant Stem bark extract Manilkara zapota had the highest concentration and Manilkara hexandra had the lowest concentration of total Alkaloids i.e., 2823µg/mg and 2673µg/mg respectively. The higher solubility of alkaloid in polar solvents provides a higher concentration of these compounds in the extracts obtained using polar solvents for the extraction. Manilkara hexandra Stem bark having highest concentration and Manilkara zapota having lowest concentration of Tannin i.e., 616µg/mg and 594µg/mg respectively. Madhuka indica stem bark had higher concentration and Manilkara zapota stem bark had lower concentration of Steroid i.e., 1038µg/mg and 916µg/mg respectively.

Madhuka indica leaves had higher concentration and Manilkara hexandra had lower concentration of Alkaloid i.e., 2750µg/mg and 2145µg/mg respectively. Madhuka indica leaves contain 296µg/mg Tannin. Steroid absent in all the plant leaves. All the standard graphs showed that strong positive linear correlation (r) which is close to +1. These graphs indicate that as the value of concentration increases, value for absorbance also increase.

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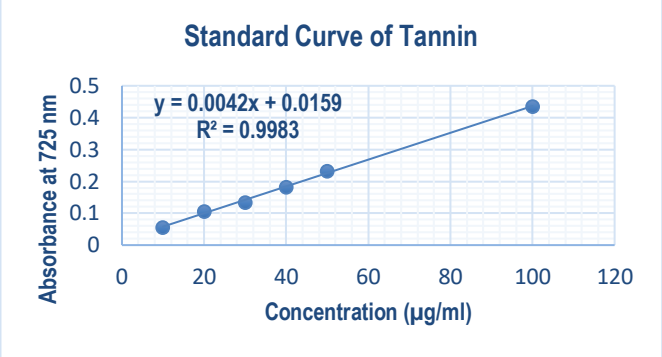

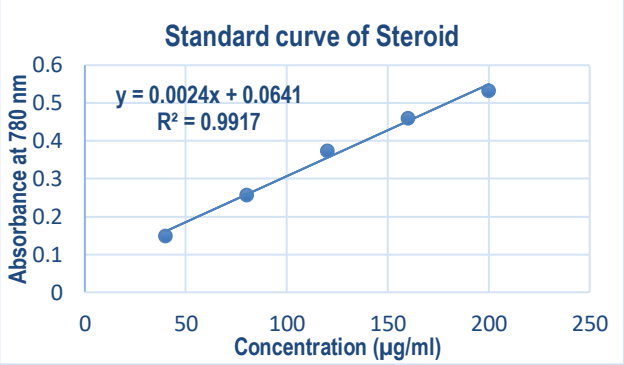

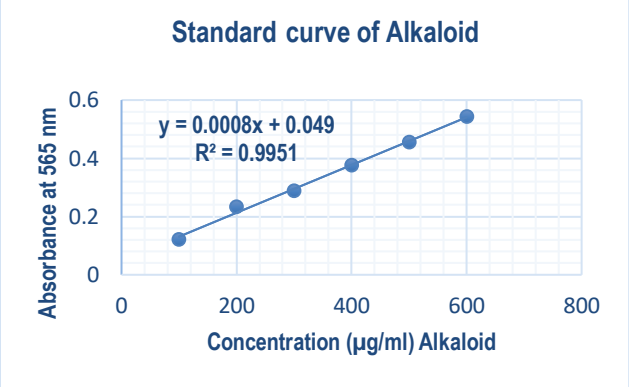

Flavonoids are plant secondary metabolites widely distributed in the plants and more than 6000 flavonoids have been identified in plants. This compound possesses a broad spectrum of chemical and biological activities including radical scavenging properties. Flavonoids are essential in human diet and are present in plant extracts that have been used for medicinal purpose. The Alkaloid are one of the most diverse group of secondary metabolites found in living organism and have an array of structure types, biosynthetic pathways and pharmacological activities. Saponin cause the leakage of protein and degradation of cell wall of enzymes from the cell.

CONCLUSION

It can be concluded that the source of secondary metabolites like flavonoid, carbohydrate, glycoside, alkaloid, tannin, terpenoid, steroid, saponin are present in the selected medicinal plants which are used in Gujarat. Because of the presence of these secondary metabolites the selected medicinal plants have high healing potential. These phytochemicals render the medicinal values of the studied plants.

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