



Qualitative analysis of ethanol and chloroform extract of selected medicinal plants.

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Abstract: Medicinal plants have bioactive compounds which are used for curing various human diseases. The phytochemical analysis is important commercially as used by pharmaceutical companies for the production of the new drugs. Phytochemicals are primary and secondary constituents. Primary constituents have chlorophyll, protein, sugar, and amino acid. Secondary constituents contain terpenoids and alkaloids. Medicinal plants have antifungal, antibacterial, anti-inflammatory, antidiabetic and anticancer activities. The present study involves four different medicinal plants *Eugenia jambolana*, *Cissus quadrangularis*, *Cassia auriculata*, and *Gymnema Sylvestre*. The selected medicinal plants have been used for treating various diseases. The main objective of the study is to find out the phytochemical constituents of the selected medicinal plants. The result shows the presence of carbohydrates, tannins, glycosides, amino acids, protein, steroids in selected medicinal plants. Polar and non-polar solvents, Ethanol, and chloroform were used for extraction.

Keywords - Medicinal plants, phytochemical, antidiabetic, anticancer, anti-inflammatory, antifungal.

I. INTRODUCTION

Nature has been a source of medicine for thousands of years and an impressive number of modern drugs have been isolated from natural sources, they are used in traditional medicine. (Anjali.D.Ruikar, 2009). Plants have a 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 (Mallikharjuna, et al., 2007). The synthesized aromatic substances (Metabolites) are used by plants as defensive molecules against predation by microorganisms, insects, and herbivores. However, some of these may involve plant odour (terpenoids), pigmentation (tannins and quinines), and flavor (Capsaicin) (Mallikharjuna, et al., 2007). However, these defensive molecules give plants their medicinal value and also used by human beings because of their great importance in the health care of individuals and communities.

Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Ahmed, L.1998). Over 50% of all modern clinical drugs of natural product origin and natural clinical products play an important role in drug development programs in the pharmaceutical industries.

Phytochemical investigations of crude plant extracts show the presence of active principles in the plant parts like bark, leaves, flowers, roots, fruits, seeds. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plants produce chemicals to protect themselves but research works demonstrate that many phytochemicals can protect humans against diseases. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. In the present work, the qualitative phytochemical analysis was carried out in different medicinal plants

Eugenia jambolana

Eugenia jambolana (E. jambolana) Lam. commonly known as Jamun or black plum is an integral part of the indigenous medicine system of India to treat various diseases. Traditionally all parts of Jamun such as fruits, leaves, seeds and bark are used in Ayurvedic medicine. Jamun plant has been reported for a wide range of medicinal properties such as antioxidant, anti-inflammatory, neuropsychopharmacological, anti-microbial, anti-HIV, anti-diarrheal, antifertility, gastro protective, antiulcer and radio-protective activities (Sagrawat H, et al., 2006)

The pulp of the Jamun berry contains anthocyanin, delphinidin, petunidin, and malvidin-glucosides, which impart its bright purple color. Both the fruit pulp and seed extracts of the Jamun berry have a long history of medicinal use and they have been extensively studied for their anti-diabetic properties. Despite a growing body of evidence supporting the anticancer properties of anthocyanin-rich berry extracts (Sharma B, et al., 2008)

Cissus quadrangularis It is a shrub, which has a slender, fleshy fibrous, smooth stem, with four winged internodes. Three variants on morphological characters occur; square - stemmed, round-stemmed, and flat stemmed. The stem and root portion of this plant possess antioxidant, antidiabetic, and antimicrobial activity. *C. quadrangularis* contain a high amount of anabolic steroidal substances, calcium, and phosphorous (Justin SR, et al., 2011) It is one of the most commonly used in traditional Ayurveda medicine. *Cissus quadrangularis* is commonly used for bone health and weight loss. It is used also in conditions such as antidiabetic, high cholesterol, and hemorrhoids. The whole plant is used in oral re-hydration, while the leaf, stem, and root extracts of this plant are important in the management of various ailments. Earlier works on *Cissus quadrangularis* report its effectiveness on the management of obesity and complications associated with metabolic syndrome (Oben J et al., 2006), as well as its antioxidant and free radical scavenging activity (Gabriel Agbor et al., 2006). The presence of β -sitosterol, δ -amyrin, δ -amyrone, and flavonoids (quercetin) has also been reported (Mehta M et al., 2001, 2000) all these components having potentially different metabolic and physiologic effects.

Cassia auriculata commonly known as Tanner's is one such herb, profoundly used in Ayurvedic medicine, known locally as 'Avaram' and belonging to the family Caesalpiniaceae. *Cassia auriculata* is a shrub with smooth brown bark and is a common plant in Asia, India, and Sri Lanka. The leaves are anthelmintic, good for ulcers, leprosy, and skin diseases. The flowers are used in urinary discharges, diabetes, and also for throat infections. (Kappers IF, et al., 2005). The seed is used in diabetes, dysentery, and chronic conjunctivitis. The bark is considered an astringent. *Cassia auriculata* used for antidiabetic, pink eye, joint and muscle pain, constipation. *C. auriculata* might decrease blood sugar. Diabetes medication is also used to lower blood sugar. Taking *cassia auriculata* along with diabetes medications might cause blood sugar to go too low

Gymnema Sylvestre (GS), a woody vine-like climbing plant, is well known in Indian traditional medicine "Ayurveda" 'It grows in the tropical forest of central and southern India. The leaves are used mostly as antiviral, diuretic, ant allergic, hypoglycemic, hypolipidemic, antibiotic, ant analgesic, and ant rheumatic agents (Baynes JW, 1999). The leaves of GS contain triterpenoid saponins belonging to the oleanane and dammarane classes. Twenty different saponins and glycosides have been reported in *Gymnema Sylvestre*. Several studies suggest that *Gymnema* acids may act as antidiabetic by promoting regeneration of islet cells and increase insulin secretion (Iwu, M.M, et al., 1999). Also, *Gymnema* acids have been reported to inhibit glucose absorption from the intestine and utilize glucose by enhancing the activities of an enzyme in insulin-dependent pathways (Justin SR, et al., 2011)

MATERIALS AND METHOD

STERILIZATION OF GLASSWARE:

Glassware were soaked overnight in cleaning solution and washed thoroughly with running tap water. Then cleaned with detergent solution and rinsed several times with tap water and finally in distilled water and air dried.

PLANT MATERIAL:

The present study included plant species *Eugenia jambolana*, *Cissus quadrangularis*, *Cassia auriculata*, *Gymnema Sylvestre*.

CHEMICALS:

90% Ethanol, Chloroform, Molisch's reagent, concentrated Sulphuric acid, 2N Sodium Hydroxide, 10% Ammonium solution, 40% Sodium Hydroxide, 0.2% Ninhydrin reagent, 2% Hydrochloric acid, 10% Sodium Hydroxide, Distilled water.

COLLECTION OF PLANTS

Four medicinal plants were collected locally from the farmlands of the Vellore area (Tamilnadu). The collected plants were identified. Fresh and tender leaves, seed, stem, flower of selected plants were used for phytochemical analysis.

PREPARATION OF PLANTS

The selected parts were removed from the plants washed under running tap water to remove dust. The sample were dried thoroughly under the shade and powdered mechanically and sieved through No.20 mesh sieve. The finely powdered leaves, flowers, stem, seed were kept in an airtight container until the time of use.

Ethanol extract: 5g of each sample was soaked in 100 ml of 95% ethanol at room temperature for 72 h. The extract was filtered using Whatmann filter paper, filtrates were collected and stored at room temperature for further study.

Chloroform extract: 5g of each sample was soaked in 100 ml of chloroform at room temperature for 72 h. The extract was filtered using Whatmann filter paper. The filtered were collected and stored at room temperature for further study.

PHYTOCHEMICAL SCREENING

TEST FOR CARBOHYDRATES:

2ml of each extract was treated with 1 ml of Molisch's reagent and a few drops of Conc Sulphuric acid. The appearance of reddish color indicate, the presence of carbohydrate

TEST FOR SAPONINS:

1ml of each extract, 1 ml distilled water was added and shaken in a graduated cylinder for 15 min. 1cm layer of foam that indicated the presence of saponins.

TEST FOR FLAVONOIDS:

2ml of each extract, 1ml of 2N sodium hydroxide was added. The appearance of yellow color indicates the presence of flavonoids.

TEST FOR GLYCOSIDES:

2 ml of each extract, 3ml of chloroform, and 10% ammonium solution were added. The appearance of the pink color presence of flavonoids.

TEST FOR TERPENOID:

1 ml of each extract, 2ml of chloroform and conc sulphuric acid, and the appearance of the red-brown color indicate presence of terpenoids.

TEST FOR PROTEIN:

2ml of each extract, 1ml of 40% sodium hydroxide, and few drops of 1% copper sulfate were added, the appearance of violet color indicates the presence of peptide.

TEST FOR COUMARINS:

1ml of 10% sodium hydroxide was added to 1ml of each extract. The appearance of yellow color indicates the presence of coumarins.

NINHYDRIN TEST:

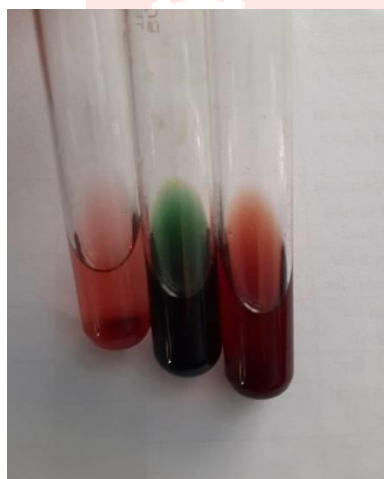
2ml of each extract a few drops of 0.2% Ninhydrin reagent were added and heated for 5 min. The appearance of blue color indicates the presence of amino acids.

TEST FOR STEROIDS:

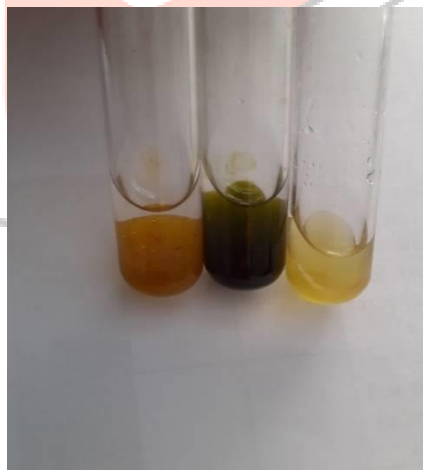
1ml of each extract, 1ml of chloroform is added and few drops of conc sulphuric acid added. The appearance of the brown ring indicate the presence of steroids.

TEST FOR PHLOBOTANNINS:

Few drops of 2% hydrochloric acid was added to 1ml of each extract. The appearance of red color indicates the presence of phlobotannins.

RESULTS

B C D
Carbohydrate



A B C
Coumarins

Qualitative phytochemical analysis: The phytochemical qualitative analysis of four plants extract from different parts of the plant confirmed the presence of carbohydrates, flavonoids, proteins, saponins, terpenoids, and Steroids. (Table 1)

TABLE 1: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF ETHANOL

Plants	<i>Eugenia jambolana</i>	<i>Cissus quadrangularis</i>	<i>Cassia auriculata</i>	<i>Gymnema sylvestre</i>
	Ethanol	Ethanol	Ethanol	Ethanol
Carbohydrate	+	-	+	+
Saponins	+	+	+	+
Flavonoids	-	+	+	+
Glycosides	+	+	+	+
Terpenoids	+	+	+	-
Protein	+	+	+	+
Coumarins	+	+	-	+
Amino Acid	+	-	-	-
Steroids	+	-	-	-
Pholobatinnis	+	-	+	-

Qualitative phytochemical analysis of ethanol extract from different parts of the plants confirmed the presence of carbohydrates, flavonoids, proteins, saponins, terpenoids, and Steroids.

TABLE 2: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF CHLOROFORM

Plants	<i>Eugenia jambolana</i>	<i>Cissus quadrangularis</i>	<i>Cassia auriculata</i>	<i>Gymnema sylvestre</i>
	Chloroform	Chloroform	Chloroform	Chloroform
Carbohydrate	+	+	+	+
Saponins	+	+	+	+
Flavonoids	-	-	+	+
Glycosides	-	-	-	-
Terpenoids	-	-	+	-
Protein	-	-	-	-
Coumarins	-	-	+	-
Amino Acid	-	-	-	+
Steroids	-	-	-	+
Pholobatinnis	-	-	-	-

The phytochemical qualitative analysis of four plants extract from different parts of the plants confirmed the presence of carbohydrates, flavonoids, proteins, saponins, terpenoids, and Steroids. Absence of Pholobatinnis.

DISCUSSION:

The preliminary phytochemical tests are helpful in finding chemical constituents in the plant material that may lead to their qualitative analysis and also in locating the source of pharmacologically active chemical compound. The qualitative analysis of bioactive compounds for the two extracts have been analyzed in this study and there is wide range of phytochemical compounds present in the two extracts as shown in table 1. The chloroform being highly nonpolar in nature was able to extract very less compound characterized like phenols, steroids and tannins. Ethanol extract and chloroform extract was found to have a wide range of bioactive compounds like alkaloids, carbohydrates, coumarins, flavonoids, proteins, phenols, reducing sugars, steroids and tannins.

Ethanol has the nature of breaking down the phytochemical Compounds of plant material .The present study revealed the presence of several phytochemical compounds like saponins, tannins and steroids. The ethanol extract revealed the presence of maximum number of bioactive compounds such as carbohydrates, proteins, Flavonoids, Saponins and coumarins. Similarly carbohydrates, proteins, amino acids were present in all four extracts. These phytochemical compounds may contribute as useful source of herbal and Ayurvedic pathway for effective treatment of various diseases considering its tremendous potential

pharmacological activities. From the above information it is clear that *E.jambolana*, *C. quadrangularis*, *C.auriculata* and *G.sylvestre* possesses potential antidiabetic, anticancer, anti-inflammatory compounds which are of utmost importance and is therefore imperative to further investigation.

The phytochemical analysis of *Eugenia jambolana* revealed the presence of Terpenoids, saponins, phenols, flavonoids, tannins and coumarins. The study helps in the identification of original drug in future. The flavonoids content showed the most representative concentration in the extracts and in the flavonoid fractions evaluated. These compounds are secondary metabolites of plant origin recognized for several biological activities (Kumar S,et al.,2013) *E. jambolana* species also presented an expressive concentration of flavonoids in its composition. The presence of several chemical constituents in the *Eugenia* samples, such as phenolic acids were observed, and flavonoids including saponins, tannins in the extract and the fractions were found, through the chemical analysis performed (Sobral-Souza CE, et al .,2014)

Cissus quadrangularis is one such species which is not only known for its nutritional value but also various other diseases and their root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil were used for various ailments in the indigenous system of medicine for treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders (Morimitsu et al., 2000). In the present study secondary metabolites were isolated from *Cissus quadrangularis* using different solvents showed the present of carbohydrate, Saponins, Flavonoids, Terpenoids, Glycosides, Protein, coumarins and steroid. Terpenoids, one of the secondary metabolite compounds present in different parts of the plant. This type of compound exists as phytoalexin in the plants and it was involved in the defence mechanism against pathogens (McCaskill and Croteau, 1998). The medicinal property of the Saponins includes antioxidant, anticancer, antifungal and antiviral (Takechi, et al., 1991).

Earlier reports showed the presence of flavonoids in methanol leaf extract of *C.auriculata* absence in chloroform (Sachin C, et al.,2014) The screening of the extract indicated the presence of Carbohydrate, Flavonoids and saponins in the chloroform extracts of leaves. Preliminary quantities of phytochemical screening of ethanol extract of *Cassia auriculata* revealed the presence of flavonoids and terpenoids compounds which are essential to prevent diseases. (Sachin C, et al.,2014)

Gymnema sylvestre exhibits rich alkaloids in all solvent extract flavonoids found only in ethanol extracts in all plants. Saponins were noticed in ethanol and chloroform extracts of *Gymnema sylvestre*. Flavonoids and phenols in general are highly effective in scavenging free radical and providing antioxidant defense in living cells.

CONCLUSION:

Now a days herbs are extensively used for the research purpose and it possesses more than one chemical entity so it has been widely used for the research investigations. The plant based compounds have the effective dosage response and minimal side effects when compared to the synthetic compounds. Phytochemical screening of *Eugenia jambolana*, *Cissus quadrangularis*, *Cassia auriculata*, *Gymnema sylvestre* leaves reveals it as a valuable medicinal plant with numerous medicinal properties. Medicinal plants have the great therapeutic and economic values in all over the world. The present study showed that ethanol extract of *Eugenia jambolana*, *Cissus quadrangularis*, *Cassia auriculata*, *Gymnema sylvestre* is rich in basic nutrients. Qualitative phytochemical screening showed it is abundant in phytochemicals such as carbohydrates, Phenoids, flavonoids, proteins, tannins, terpenoids, and glycosides especially it was found in high amounts in ethanol extract than chloroform. From the study, it may be concluded ethanol extract of four different parts of plants acts as the potential source of phytochemicals that may be used in traditional medicine for preventing diseases such as antidiabetic, anticancer, anti-inflammatory, antibacterial, antifungal.

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