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Screening of Phytochemicals in Ethanolic Extract of *Momordica charantia* leaves and *Semicarpus anacardium* nuts

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ABSTRACT: *Momordica charantia* and *Semicarpus anacardium* were used since ancient for many medicinal purposes in traditional medicine system. *Momordica charantia* is a well-known plant, found in Asia including India, it has a wide range of pharmacological activities so that the parts of these plant are used as treatment modalities in many diseases as a folk remedy. The phytochemical composition of locally found *Momordica charantia* and *Semicarpus anacardium* was investigated using standard analytical methods. Phytochemicals like carbohydrates, tannin, flavonoids, phenolic compounds, alkaloids, saponins, steroids etc. were found present/absent. The study indicates phytochemicals present in these plant parts maintained the medicinal value of these plants.

Keywords: *Momordica charantia, Semicarpus anacardium* Saponin, Alkaloid, Polyphenol, Flavonoid

I. INTRODUCTION

Identification of phytochemicals present in plants is the most important tool to know the active moiety of plants [1]. *Momordica charantia* is commonly known as Bitter melon, is a member of the family Cucurbitaceae. The Latin name *Momordica*, it is also referred to by diverse names around the world like balsam pear (English), Karella (Hindi or Urdu), Nigauri or Goya (Japanese), Kho qua (Vietnamese), Koguai (Taiwanese), Assorossie (French) and Ampalaya (Philippines) [2]. Bitter melon is extensively cultivated in tropical and subtropical countries and used as a dietary product and also popular as a traditional medicinal plant. A lot of scientific literature tell us that Bitter melon has been linked with a wide range of therapeutic effect, such as, anti-viral [3], anticancer [4], hypolipidaemic [5], anti-inflammatory [6], immuno-modulatory [7] hypocholesterolaemic [5], and anti-diabetic [8] properties. Studies have reported that different phytochemical contents are present in different varieties of bitter melon. However, many proposed therapeutic effects are due to the presence of its flavonoid, phenolic, alkaloid contents [9]. Previously we have evaluated phytochemicals in *Momordica charantia* fruit juice [10].

The second plant used in this study for phytochemical screening is *Semecarpus anacardium*, a marking nut tree. It is commonly known as bhallataka, belongs to family Anacardiaceae. This plant is generally used in Ayurveda and Unani fields as herbal drug and used in a cough, anorexia, indigestion, asthma, piles, ulcer, and various nervous diseases [11, 12]. Presence of Phenolic compounds, biflavonoids [13, 14] bhilawanols [15] anacardic acid [16] alkenyl catecholsare confirmed by other phytochemical studies. The nut milk extract possesses several biological activities such as anti-arthritis [17], antispermatogenic [18], antimicrobial [19, 20] and mutagenic properties [21]. Nuts of this plant possess various therapeutic properties and are generally used in the Ayurveda and Siddha systems of medicine [22]. Therefore, we have selected leaves of *Momordica charantia* and nuts of *Semicarpus anacardium* locally collected.

II. MATERIALS AND METHODS

A. Collection of plant parts

Different parts of each plant material were collected at different times, localities and conditions. Fresh cultivated leaves of *Momordica charantia* and dried nuts of *Semicarpus anacardium* were used in the present study.

Momordica charantia. The fresh cultivated *Momordica charantia* plant leaves were collected from the Agricultural field nearby Lucknow, India and identified by Prof. S. Lavania, Department of Botany, University of Lucknow, Lucknow, India. A reference specimen (voucher No. LWU-2016-1) has been deposited in the herbarium of Department of Botany, University of Lucknow, Lucknow, India.

***Semicarpus anacardium*.** The dried nuts of plant *Semicarpus anacardium* was purchased from the local market of Lucknow, India and identified by Prof. S. Lavania, Department of Botany, University of Lucknow, Lucknow, India. A reference specimen (voucher No. LWU-2016-2) has been deposited in the herbarium of Department of Botany, University of Lucknow, Lucknow, India.

B. Preparation of Plant Extracts

I. *Momordica charantia* (MC) leaves extract: Leaves of MC were washed and shed dried. Dried leaves were powdered and then extracted in absolute alcohol using soxhlet apparatus.

II. ***Semicarpus anacardium* (SA) nuts extract:** Nuts of SA were washed, dried and macerated and then extracted in absolute alcohol with the help of soxhlet extraction apparatus.

C. Preliminary Phytochemical Screening

The extracts of MC, SA were used for preliminary screening of phytochemicals. The various standard procedures were used for the phytochemical analysis such as carbohydrates (Molisch's test), reducing sugars (Fehling's test), combined reducing sugars (Fehling's test), tanins, alkaloid (Mayer's test and Hager's test), flavonoids (Alkaline reagent test and Shinoda test), phenolic compounds (Shinoda test and Ferric chloride test), steroids (Liebermann-Burchard test), terpenoids (Salkowsi's test), saponins, and soluble starch [23, 24, 25].

D. Phytochemical analysis

Test for carbohydrates

Molisch's test for carbohydrates. To the 2 ml of extract, few drops of Molisch's reagent was added and then shaken well. 2 ml of the con. H_2SO_4 was added on the sides of the test tube. The Mixture was then allowed to stand for two minutes. Formation of a red or dull violet ring appeared at the interphase of two layers indicated the presence of carbohydrates.

Barfoed's test for monosaccharides. To the 1 ml of extract, 1 ml of Barfoed's reagent was added and then heated in a water bath for 2 minutes. The reddish precipitate was considered as a positive test for monosaccharides.

Fehling's test for free reducing sugars. Few drops of test extract were heated with 5 ml of equal volumes of Fehling's solutions A and B. Formation of red precipitate indicated the presence of free reducing sugars.

Test for alkaloids

Mayer's test. To the 2-3 ml of extract, 1% aqueous HCl and few drops of Mayer's reagent (Potassium mercuric iodide solution) was added, the occurrence of creamy white precipitate was an indication for the presence of alkaloids.

Hager's test. To the 2-3 ml of extract, few drops of Hager's reagent (saturated solution of Picric acid) was added, the appearance of yellow precipitate was an indication for the presence of alkaloids.

Test for flavonoids

Shinoda's test. To the extract, few fragments of Magnesium ribbon was added and then HCl was added drop wise, pink, orange or red to purple color was appear that indicate the presence of flavonoids.

Ferric chloride test. To the 2 ml of extract, few drops of 10% ferric chloride were added. A green-blue or violet coloration indicated the presence of flavonoids.

Sodium hydroxide test. To the extract, 2ml of 10% aqueous sodium hydroxide was added, yellow color was developed, yellow color disappeared after addition of dilute HCl, indicated the presence of Flavonoids.

Test for phenolic compounds

Ferric chloride test. To the extract, few drops of $FeCl_3$ was added, the appearance of blue-green color indicated the presence of phenols.

Shinoda test. To the extract, few fragments of Magnesium ribbon and conc. HCl was added drop wise. Yellowish, yellow-orange or orange color appears after few minutes.

Test for terpenoids

To the 5 ml of extract, 2 ml of chloroform and 3 ml of conc. H_2SO_4 were added, a monolayer of reddish brown color formed at the interface indicated the presence of terpenoids.

Liebermann-Burchard test for steroids. To 0.5 g of ethanolic extract, 2 ml of acetic anhydride was added, the solution was cooled well in ice after that 2 ml of conc. H_2SO_4 was added carefully. The color change from violet to blue or bluish- green indicated the presence of steroids.

Test for saponins. The extract with 5 ml of distilled water was shaken vigorously for about 5 minutes. The formation of a 1cm layer of foam appeared which persisted on warming indicated the presence of saponins.

Test for soluble starch. To the extract, 1ml of 5% KOH was added, boiled, cooled and then acidified with H_2SO_4. Yellow color appeared, indicated the presence of soluble starch.

Test for tannins. To the 2 ml of extract, few drops of 1% $FeCl_3$ was added, the occurrence of a blue-black, green or blue-green precipitate indicated the presence of tannins.

III. RESULTS AND DISCUSSION

The results demonstrated the presence of several phytochemicals in the extract of plants (Table). Leaves extract of *Momordica charantia* contains soluble starch, flavonoids, phenols, steroids, alkaloids, tannins and, saponins. These phytochemicals exhibit several biological functions, in a mouse mammary tumor model [26] and in a rat colonic, aberrant crypt foci model [27] have shown anticancerous activity using seed or fruit extracts of bitter melon. In another study thirteen cucurbitane-type triterpene glycosides, out of which eight new compounds named charantosides I, II, III, IV, V, VI, VII, and VIII and five known compounds have been isolated from a methanol extract of the fruits of Japanese *Momordica charantia*. [28].

Nuts of *Semicarpus anacardium* contain carbohydrates, flavonoids, phenolic compounds, steroids, and tannins. The flavonoids, steroids, phenols, and alkaloids are important phytoconstituents which effect the proliferation of cells and inhibit apoptosis by intrinsic and extrinsic pathway in cancer cells.

These compounds may act as an antimicrobial activity and inhibit resistance of the microorganism to conventional drugs is increases day by day. These natural medicinal plants containing phytochemicals could be a new efficient and cost-effective ways has necessitated for the control of infectious diseases. The effectiveness of traditional herbs against microorganisms is shown by many reports so that plants have become one of the bases of modern medicine [29]. Another study demonstrates that phytochemicals like terpenes, Flavonoids, Phenolics, Saponins, Alkaloids, and glycosides are present in the Petroleum ether solvent nut extract of the *S. anacardium* and possess *in vitro* anti-microbial activity [30].

Weaver *et al*., 2012 [31] reported that flavonoid consumption has a stronger association with bone. Some flavonoids enhance the bone formation and some flavonoids inhibit bone resorption which ultimately effects the osteoblast and osteoclast differentiation.

According to Cicerale *et al*., 2010 [32] some physiological parameters, such as platelet, plasma lipoproteins, inflammatory markers, oxidative damage, cellular function, antimicrobial activity and bone health are positively affected by phenolic compounds.

Some phenolic compounds like verbascoside, thymoquinone, resveratrol, mangiferinand some flavonoids have been found to prevent periodontal tissues damage, a disease that affects gum and bones, in animal models. Some *in vitro* studies and some animal experiments show that these phenolic and flavonoid compounds have protective effects like antioxidative properties, oxygen and nitrogen scavenging abilities, and also these compounds have inhibitory effects on cell signaling pathways that are related to inflammatory processes that effect on ROS or RNS production as well as on antioxidant defense systems [33].

It was found that phytoestrogens, saponins, and flavonoids like xanthohumol and kaempferol enhance the osteogenic differentiation pathway by stimulating the osteogenic transcription factors Runx2 and Sp7 while these compounds adversely affects the process of adipogenic differentiation [34].

Studies done by Pandey and Rizvi, 2009 [35] suggested that diet rich in plant polyphenols protects against the development of cardiovascular diseases, cancer, osteoporosis, diabetes and neurodegenerative diseases.

According to Somjen *et al*., 2011 [36] phytoestrogenic compounds and their synthetic analogs stimulates cultured bone cells in hyperglycemic conditions.

Table 1: Phytochemical investigation of ethanolic extract of MC and SA.

S. No.	Constituents	Test	Results	
			MC	SA
1	Carbohydrates	Molisch’s test	_	+
		Barfoed’s test	_	+
		Fehling’s test	_	+
2	Soluble starch		+	_
3	Flavonoids	Shinoda’s test	_	+
		Ferric chloride test	_	+
		Sodium hydroxide test	+	+
4	Phenols	Shinoda’s test	+	_
		Ferric chloride test	+	_
5	Steroids	Liebermann-Burchardtest	+	+
		Salkowski’s test	+	+
6	Terpenoids		_	_
7	Alkaloids	Mayer’s test	+	_
		Hager’s test	+	_
8	Tannins		+	+
9	Saponins		+	_

SA: *Semicarpus anacardium*; MC: *Momordica charantia*; +: Present; −: Absent

Purpose of this study is to identify the phytochemicals presents in the locally available *Momordica charantia* plant leaves and nuts of *Semicarpus anacardium.*
The phytochemicals present in these plants have been shown multifunctional properties, they may be used for various therapeutic purposes like antimicrobial, anti-inflamatory, antidiabetic, antiosteoporotic, anticancerous and antioxidant properties. Further studies are needed to identify and isolate phytochemicals from these medicinal plants and evaluate their medicinal properties in the treatment of some diseases like cancer, osteoporosis, diabetes, osteoarthritis and some neurodegenerative diseases.

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