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Chromatographic Evaluation of the Ethanolic Extracts of Seeds of *Momordica charantia* L.

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Abstract

Momordica charantia, commonly used in Indian sub-continent known for its medicinal properties. It belongs to the family of cucurbitaceae and widely used against many diseases since ancient times. The aim of this study was to determine the chromatographic profile of ethanolic extract of seeds of Mormordica charantia.

Key-Words: Anti- Cancer, Momordica charantia, Ethanolic Extract

Introduction

Bitter melon, *Momordica charantia* L. is a member of the Cucurbitaceae family. The latin name "Momordica"means 'to bite' and refers to the leaves of the bitter melon plants, which have jagged edges and look like they have been bitten [1]. It is also referred to by different names around the world: balsam pear (English), Karella (Hindi or Urdu), Nigauri or Goya (Japanese), Ku gua (Madarin), Ko guai (Taiwanese), Kho qua (Vietnamese), Ampalaya (Philippines) and Assorossie (French). Generally, the bitter melon fruit has an oblong cucumber-like shape, ranging from 9 to 60 cm long, but in contrast to cucumbers, it has a very warty-looking exterior [2]. Bitter melon is widely cultivated in tropical and subtropical countries, where it is a popular traditional medicinal fruit. In the scientific literature, it has been linked with a wide range of therapeutic effects, including anticancer [3], anti-viral [4], anti-inflammatory [5], hypolipidaemic [6], hypocholesterolaemic [6], immuno-modulatory [7] and anti-diabetic [8] properties. Studies have reported that different varieties of bitter melon may differin their content of bioactive compounds [9,10].

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However, some of the proposed therapeutic effects have been inpart attributed to its content of flavonoids [7,8,11]. Flavonoids occur naturally in plants either linked to sugars (glycosides) or without the sugars (aglycones).Flavonoid aglycones are usually extracted with less polar solvents, such as benzene, chloroform and diethyl ether [13] while flavonoid glycosides are commonly extracted with more polar solvents, such as acetone, butanol, methanol and ethanol [14-16]. Ethanol [11,17], water [11] and methanol [18] have been used for extracting flavonoids and other bioactive compounds from bitter melon. However, studies on the use of different solvents, including water and less polar organic solvents, for the extraction of flavonoids from different bitter melon varieties are still limited. For example, the ratio of flavonoid aglycones to glycosides may differ between varieties and thereby lead to different extraction efficiencies, depending on the type of solvent used [13-16]. Herbal products seem to be an unexplored domain whereby a large number of pharmaco-therapeutic agents can be isolated and screened to determine therapeutic activity. *Bitter melon* is one of the widely used herbs, used as condiment in India; the plant is reported to have antidiabetic, analgesic, anticancer potential.The drug is used as insulin deficiency aphrodisiac, tonic, diuretic, expectorant, Diabeties, and useful in inflammation, Ulcer, cancer pains, Microbes, sour eructation's, tuberculosis, diseases of kidney catarrhal affections to destroy bad smell in mouth and other part of the body. Therefore, the aim of the current work is to determine chromatographic profile of the ethanolic extract of selected plant.

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Material and Methods

Plant Materials

The fresh whole plant of *bitter melon* was purchased from local market. It was then identified and authenticated by Dr. A.B. Tiwari, Sr. Scientist, Department of Crop and Herbal Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P).

Extraction

The plant was shade dried at room temperature and then ground into fine powder. The powder was sieved to have a uniform size. 25 gm of dried powder was extracted with different solvents viz, pet. ether, benzene, chloroform, acetone, ethanol and finally with water in sohxlet apparatus. The extract was filtered using a whatman filter paper no. 4 and concentrated at 40°C; dried extract was refrigerated at 4 °C until use. The extract was concentrated and dried to a constant weight. The dried extracts of the drugs were evaluated for physical parameters such as consistency, color, odor and taste. The presence of desired phytochemicals i.e. flavonoids, tannin. Qualitative chemical tests were carried out for the ethanolic extract of *Bitter melon*..The results of the tests showed the presence of carbohydrates, reducing sugars, saponins, phenolics, tannins, and flavonoids.

Chemicals

Methanol and acetone were obtained from Merck Company. Ethanol was purchased from Fronine and the n-Butanol Sodium nitrite, aluminium chloride, sodium hydroxide and rutin were purchased fromSigma-Aldrich Company pvt.ltd

TLC Profile

The solvent system was poured to a depth of 0.5 cm in a rectangular chromatographic glass chamber. The chamber was lined with a piece of filter paper to ensure proper saturation. The spots of extract were applied on a silica gel-G plate with the help of capillary tube. The distance between two spots was kept approximately 2.0 cm. The applied spots were dried at room temperature and the plate was gently placed inside the glass chamber. The angle of the plate with the vertical was kept approximately 15°. The chromatogram was developed till the solvent front migrated to about 10.0 cm. The plate was taken out and the solvent front was marked. The plate was dried at room temperature and inspected either under UV light or sprayed with the specific detecting reagent. The colored spots were marked and the Rf value of each separated component was calculated and best resolution was obtained in, Ethyl acetate: Methanol: water (100:13.5:10) Toluene: Ethyl acetate (93.5:7). The TLC plate examined under sun light showed the presence of 8 spots. The same plates were derivatized with anisaldehyde-sulphuric acid reagent and ferric chloride soln., but after using these spraying reagents none of them showed good resolution of the spots so only sun light detection was choosed for the further investigations as it showed the best resolution. Many other solvent systems were investigated before developing the best solvent system but none of those gave the satisfactory resolution or the good separation Ethyl acetate: Methanol: water (100:13.5:10) gave the same separation as by Toluene-chloroform-ethanol (40:40:20) but the resolution of the spots were not as clear the best solvent chosen.

Results and Discussion

Natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy. Thin Layer Chromatography revealed that ethanol gives better extraction of the phytochemicals than water since the ethanolic extract resolved into maximum number of bands as compare to aqueous extract. On the basis of all the qualitative chemical tests performed in extract; ethanolic extract was subjected for further phytochemical and pharmacological studies as the ehtanolic extract showed the presence of variety of desired phytochemicals. Ethanolic extract of the drug was chromatographed on TLC plates and best resolution was obtained in, Ethyl acetate: Methanol: water (100:13.5:10) Toluene: Ethyl acetate (93.5:7).. The TLC plate examined under sun light showed the presence of 8 spots. The same plates were derivatized with anisaldehyde-sulphuric acid reagent and ferric chloride soln., but after using these spraying reagents non of them showed good resolution of the spots so only sun light detection was choosed for the further investigations as it showed the best resolution.

Conclusion

TLC of ethanolic extract was done in order to separate out as many as compounds possible. After trying to many solvents and solvents system Ethyl acetate: Methanol: water (100:13.5:10), came out to be the best solvent system as it gave 8 spots in glass plate coated with silica gel G .After that we conducted the Co-TLC of ethanolic extract.

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Table I: TLC studies of ethanol extract of bitter melon

S.No.	Mobile Phase	Detector	Observation	Possible Phytoconstituents
1.	Ethyl acetate: Methanol: water (100:13.5:10)	Vanillin sulphuric acid	Red/yellow/blue-green	Bitter principle
		Dragendorffs reagent	Orange , Red	Alkaloid
		NP/PEG and UV	Yellow/green/orange	Flavonoid
2.	Toluene:Ethyl acetate:Water (93.5:7)	VS reagent	Red/ yellow/Brown/Blue-green	Essential oil
		NH3/KOH	Light Blue brown	Coumarin

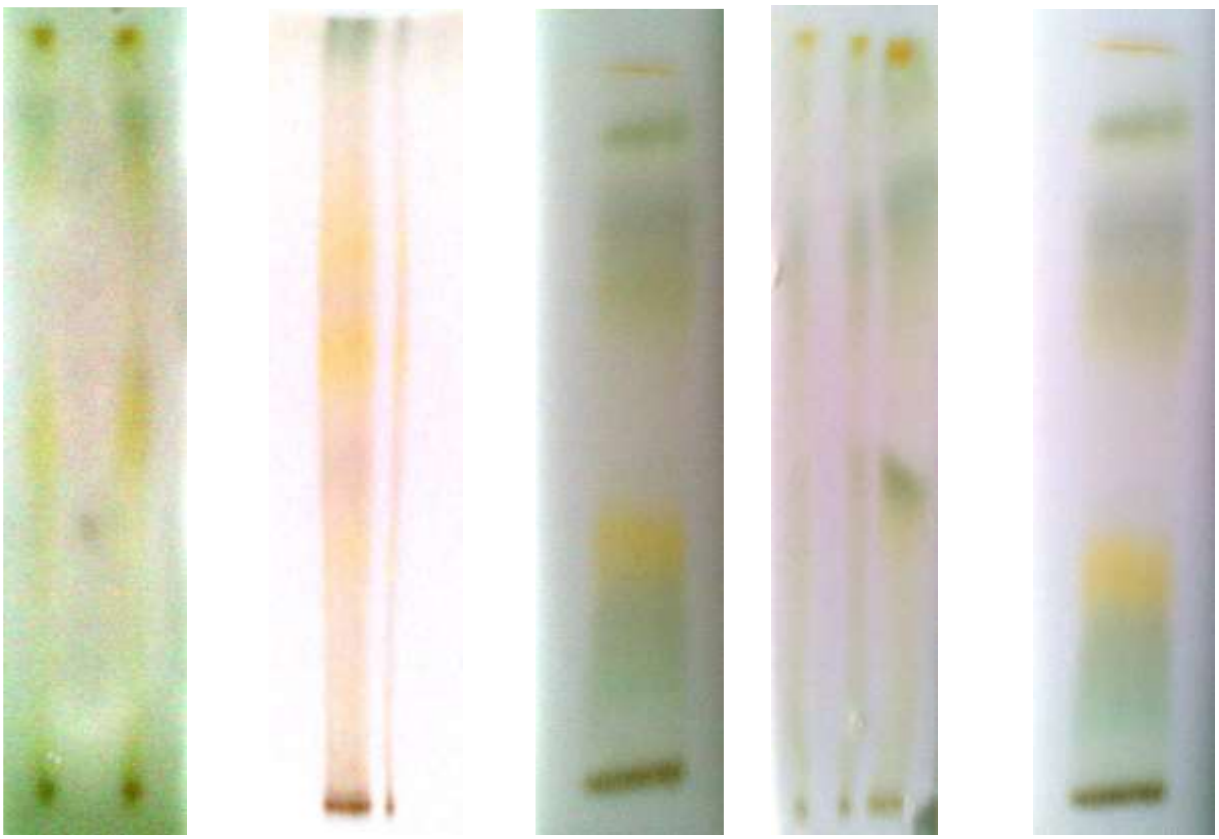

Fig. 1: Chromatographic analysis

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