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Research Article

Phytochemical Screening and Determination of Total Phenolic Content of *Citrullus colocynthis* Linn

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Abstract

Citrullus colocynthis (L) Schard (Cucurbitaceae) is commonly known as "Indrayan or Bitter Apple "distributed throughout the tropics. The ethnobotanical uses of this plant include its use as an abortifacient, cathartic, purgative and vermifuse. Phytochemical screening was carried out by using standard procedures. Steroids, alkaloids, flavonoids, carbohydrates, proteins, glycosides, saponins, amino acids and phenolic compounds were detected in Petroleum ether, Ethanol and Aqueous extract of *C. colocynthis*. Total Phenolic content determination by using spectrophotometric method and that was found to be 3.2310 g; 3.3370 g of Gallic acid is equivalent to 100 g of aqueous and ethanolic extract respectively.

1. INTRODUCTION

Citrullus colocynthis Linn Schard (Cucurbitaceae) belongs to family Cucurbitaceae. This study includes preparation of different polar and nonpolar extracts by soxhlet extraction for detailed analysis. Monoecious root, Perennial Stem diffuse or creeping, slender, angled, branched, hirsute or scabrid. Tendrils are simple or 2-fid,slender,hairy.Leaves are very variable,3.8-6.3 by 2.5-5cm in the wild form ,usually deltoid in outline pale green above ashy beneath, scabrid on both surfaces. Fruit is globular, slightly depressed, 2-3inch in diameter. Seed is 0.16-0.25inch long, pale brown and not margined. Flower having hairy calyx, long and villous peduncles about 6-13mm, campanulate is 5mm long, corolla is 2mm long. Ovary is ellipsoid and densely hairy [1, 2]. Ethanolic extract (50%) shows significant anti-inflammatory activity in albino rats. The ethanolic extract of leaves and flowers exhibits antibacterial activity against a number of Gram-positive and Gram-negative bacteria [3].It is used in the treatment of Cough, Opthalmia, Neuralgia, Migraine and Bronchitis. The ethnobotanical uses of this plant include its use as an abortifacient, cathartic, purgative and vermifuse, and for the treatment of fever, cancer, amenorrhea, jaundice, leukemia, rheumatism and tumor [4].The bioactive non-nutrient phytochemicals such as phenolic compounds, are supposed to play an important role in the prevention of major chronic diseases[5].In effect, phenolic compounds are major antioxidants of our diet[6].In present study extract of *C. colocynthis* plant were studied for different phytoconstituent and total phenolic content determination.

2. MATERIALS AND METHODS

2.1 Plant material

Fresh plant *C. colocynthis* was collected from Ahmednagar district of Maharashtra in September 2009 and authenticated by Mr. S.C. Majumdar, Botanical Survey of India, Pune, where a sample specimen (Voucher number: BSI/ 501) has been deposited.

2.2 Extraction

Dried and coarsely powdered plan material of *C. colocynthis* was subjected to successive solvent extraction in Soxhlet extractor using petroleum ether, ethanol as solvent and the marc left was refluxed with water.

2.3 Drugs and Chemicals

Following chemicals were used for the study.
Chemicals: Petroleum ether (60-800C) AR, ethanol AR and tween 80.

2.4 Phytochemical Investigation

The preliminary phytochemical investigation was done by the standard chemical tests of Evans[7].

2.5 Determination of Total Phenolic Content

The concentration of phenolic in plant extracts was determined using spectrophotometric method. Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteau's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared**,** containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteau's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45^{o}C for 45 min. The absorbance was determined using spectrophotometer at λmax = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolic was read (mg/ml) from the calibration line; then the content of phenolic in extracts was expressed in terms of gallic acid equivalent (mg/g of GA/g of extract). All the tests were performed in triplicate [8].

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3. RESULTS AND DISCUSSION

3.1 Preliminary Phytochemical Test

Table 1: Preliminary phytochemical screening of various extracts of *C. colocynthis*

Sr. No.	Chemical Test	Petroleum ether Extract	Ethanol Extract	Aqueous Extract
1	Carbohydrate	-	+	+
2	Protein	-	-	-
3	Amino Acid	-	+	+
4	Glycoside	-	+	+
4	Steroid	+	+	+
5	Alkaloid	+	+	+
6	Flavonoid	+	+	+
7	Saponin	-	+	+
8	Total Phenolic compound	-	+	+

+: indicates presence of constituents, -: indicates absence of constituents

The extracts obtained after extraction were characterized by preliminary phytochemical test for rough ideas of constituents present in extract. Petroleum ether extract showed the presence of steroids, alkaloids and flavonoids. Ethanol extract showed positive test for carbohydrates, Amino acid, Steroid, flavonoids, alkaloids, glycosides, saponins and phenolic compounds. The aqueous extract showed presence of carbohydrates, amino acids, steroids, phenolic compounds, flavonoids and saponins.

3.2 Total Phenolic Content

Total phenolic content was estimated by Folin-ciocalteu method. Total content of phenolic compound was calculated as Gallic acid equivalent. The obtained observations are mentioned in table 1 and plotting graph absorbance versus concentration (in fig.1).

Table 2: Phenolic content of *C.colocynthis*

Sr. No.	Sample	Concentration	Absorbance
1.	Gallic Acid	a) 0.01	0.6987
		b) 0.02	0.8835
		c) 0.04	1.1866
		d) 0.06	1.9953
		e) 0.08	2.0025
		f) 0.1	3.1506
2.	Aqueous extract	0.0323	1.1949
3.	Ethanolic extract	0.0333	1.2242
4.	Pet-ether	0.0087	0.5469

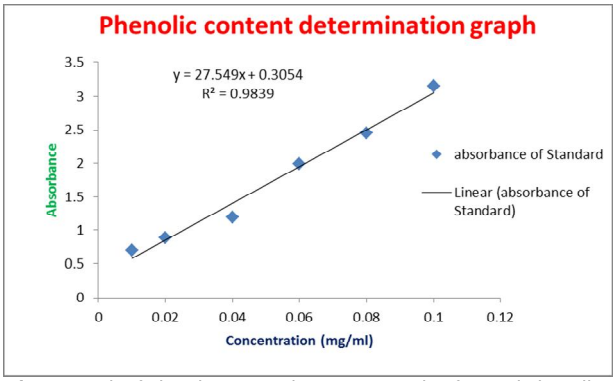

Fig.1. Graph of absorbance against concentration for total phenolic content.

Total Phenolic content was found to be 3.2310 g, 3.3370 g and 0.8703 g gallic acid equivalent per 100 g aqueous extract Ethanolic extract and pet-ether extract respectively by using the equation (y = 27.549 + 0.3054).

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