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QUALITATIVE & QUANTITATIVE ESTIMATION OF PHYTOCHEMICALS IN *GARCINIA INDICA* FRUIT RIND EXTRACT

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Abstract

Plant bioactive organic molecules have recently attained enormous importance as a result of their dynamic role and adaptive applications in the treatment of terminal diseases. This study is an attempt to elaborately qualitative & quantitates the bioactive constituents from *Garcinia indica*. The fruit rinds of plant were collected & subjected to extraction by four different solvents. Further qualitative & quantitative study was performed for all the derived extracts. From the results it was observe that & loss on drying was found to be 57%. The extractive values obtained for solvents Chloroform, Ethyl Acetate, Ethanol & Aqueous were 12.06 %, 1.91 %, 12.3 % & 2.74 %. In case of chloroform extract of *Garcinia indica* flavonoid, sterol & diterpenes are found to be present. Identical results were obtained for ethyl acetate extract. For ethanolic extract positive results were obtained for carbohydrate, flavonoid, alkaloid, tannin, glycosides, sterol, proteins & diterpenes. Aqueous extract was also enriched with various phytoconstituents except phenol, tannin, lignin, saponin. The Rf value obtained for quercetin was found to be 0.58. The Rf value obtained for all the four extracts were approximately equal to quercetin, this indicates extract contain quercetin along with other class of

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flavonoids. Total flavonoid for ethanolic, chloroform, ethyl acetate, Aqueous extract as 2.44, 0.77, 1.25 & 1.08 mg/100mg. Also, the alkaloid content was noticed to be highest in ethanolic extract which is 3.14mg/100mg, while for aqueous extract about 2.31mg/100mg of alkaloid content was estimated. From the results it can be interpreted that *Garcinia indica* contain appreciable quantity of flavonoid & alkaloid which are believed to have therapeutic applications.

Keywords: Phytochemicals, *Garcinia indica*, Total alkaloids content, Total flavonoid content, Thin layer chromatography, Medicinal plants.

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Introduction

Plants serve humans in a variety of ways, hence they are seen as a gift from nature. Since the beginning of recorded human history, their successful usage as medicine has been documented. These have been recognised as phytomedicine ingredients since the dawn of time. From plants, a wonderful variety of commercial compounds have also been extracted. These plant-based natural compounds may be isolated from a variety of plant parts, including the roots, stems, bark, leaves, fruits, flowers, seeds, and rhizomes, demonstrating that every part of the plant contains these biologically active substances. Treatments utilising medicinal plants have attracted enormous global attention in recent years (Tiwari *et al*., 2011; Arica e*t al*., 2015; Lucy & Edgar, 1999). Plant bioactive organic molecules have recently attained enormous importance as a result of their dynamic role and adaptive applications in the treatment of terminal diseases. In addition to being the most abundant bio-source of pharmaceuticals in antiquity, medicinal plants are also widely used today as food supplements, nutraceuticals, traditional medicines, pharmaceutical intermediates, and other products (Kala, 2005). Numerous factors, including growing confidence in herbal therapy, have contributed to the continued development of recognition for medicinal plants. The extraction and development of medications and chemotherapeutics from these plants as well as from commonly used herbal treatments has been linked to a growing dependence on the use of these medicinal plants in industrialised organisations (Adesokan *et al*., 2008). The anti-oxidant, anti-microbial, and antipyretic activities of the phytochemicals contained in plants may be the basis for their medicinal qualities. The World Health Organisation states that medicinal plants are the best source for a wide range of medications.

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Therefore, research into these plants is necessary to have a better understanding of their characteristics, safety procedures, and utility (Nascimento *et al.*, 2000). The plant *Garcinia indica* is a member of the Clusiaceae family and is a small to medium-sized plant. It is a common home cure for infections, heat strokes, and flatulence. Traditional medicine based on Ayurveda has detailed several of the fruit's healing properties. It can be used as an infusion to treat skin conditions like rashes brought on by allergies; to treat burns, scalds, and chaffed skin; to relieve sunstroke; as a liver tonic and appetiser; to increase appetite and quench thirst; and as a cardiotonic and for bleeding, piles, dysentery, tumours, and heart disease. The antioxidative, chelating, free radical scavenging, anticancer, anti-inflammatory, and antiulcer properties of this plant are also being explored pharmacologically (Jagtap *et al*., 2015; Chate *et al*., 2019; Parasharami *et al*., 2015).

Materials & Methods

Chemical and reagents

Potassium Mercuric Iodide, Potassium Iodide, Iodine, Ferric chloride, Lead acetate, Nitric acid, Copper acetate, Aluminum chloride Potassium Bismuth Iodide, Picric acid, Sodium nitropruside and Sodium hydroxide obtained from Loba Chemical Pvt Ltd (Mumbai, India). Hydrochloric acid, methanol and ethanol were obtained from Merck Ltd, Mumbai, India. Quercetin was purchased from Hi Media, Mumbai. All solvents and reagents were of analytical grade.

Collection of plant material

Every parts of the plant like bark, leaves, flowers, roots, fruits and seeds may contain active secondary metabolites. Fresh & healthy plant fruits rinds, free from diseases of *Garcinia indica* were collected from local market of Bhopal (M.P.) in the month of April, 2021.

Extraction by maceration process

40.0 gram of dried plant materials of fruits rinds of *Garcinia indica* was exhaustively extracted with different solvent (chloroform, ethyl acetate, ethanol and aqueous) by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts (Singh, 2008).

Phytochemical screening

The assessment for presence or absence of phytochemicals was performed by standard procedure (Savithramma *et al*., 2011).

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Estimation of total alkaloids content

For preparation of standard Atropine solution, dissolved 10 mg Atropine in 10ml methanol. Dilute the stock solution in methanol to give working standard of (40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml, 120 µg/ml). For preparation of plant extract solution, take 10 mg plant extracts and dissolved it into five ml of methanol then filter this solution and adjust the volume upto 10 ml with methanol. The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract (John *et al*., 2014).

Total phenol content estimation

The modified folin-ciocalteu method was used to calculate the extract's total phenol concentration (Parkhe and Bharti, 2019). 10 ml of methanol was used to dissolve 10 mg of gallic acid, and different aliquots of 10- 50 g/ml were created. 10 ml of methanol were added to 10 mg of dried extract before filtering. The estimate of phenol required two millilitres of this extract (1 mg/ml). 1 ml of the Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate were combined with 2 ml of the extract and each standard. The mixture was vortexed for 15 seconds before being left to stand for 10 minutes to develop the colour. In order to determine the absorbance, a spectrophotometer was used at 765 nm.

Total flavonoids content estimation

The aluminium chloride technique was used to determine the total flavonoids content. Various aliquots of 5- 25 g/ml quercetin were produced in methanol after 10 mg of quercetin had been dissolved in 10 ml of the alcohol. 10 ml of methanol were added to 10 mg of dried extract before filtering. This extract, 3 ml (1 mg/ml), was used to calculate the amount of flavonoids. 3 ml of extract of each standard were combined with 1 ml of a 2% AlCl3 solution, which was then let to stand for 15 minutes at room temperature. At 420 nm, absorbance was then measured (Kim *et al*., 2012).

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Results & Discussion

The fruit rinds of the plant were taken for the study. A first the Loss in weight on drying is calculated by subtracting Weight of plant material after drying at room temperature from Weight of plant material in wet, fresh condition. Loss on drying refers to Calculating the amount of volatile stuff present in tablets, capsules, or bulky material. So, in this case loss on drying was found to be 57 %. The dried plant material was then subjected to extraction by four different solvents namely Chloroform, Ethyl Acetate, Ethanol & Aqueous. Further percentage yield was calculated for each of the extract. The extractive values can be used to assess the chemical components of a crude medication and estimate which components are soluble in which solvents.

The identification of used-up or tampered pharmaceuticals is the main application of extractive values. The quality and purity of the medicine are determined by the extraction value of the crude substance. When evaluating crude pharmaceuticals, the extractive value that is water-soluble is very essential. Less extractive value suggests the addition of stale material, adulteration, or improper drying, storage, or formulation processes. The extractive values obtained for solvents Chloroform, Ethyl Acetate, Ethanol & Aqueous were 12.06 %, 1.91 %, 12.3 % & 2.74 %.

The phytochemical screening is then performed to know the presence or absence of bioactive principals. Phytochemical components can either be inert or therapeutically active. For the purpose of finding various categories of naturally occurring phytochemicals, numerous phytochemical surveys have been conducted. The method to phytochemical research is thought to be successful in identifying the bioactive profile of plants with therapeutic value.

In case of chloroform extract of *Garcinia indica* flavonoid, sterol & diterpenes are found to be present. Identical results were obtained for ethyl acetate extract. For ethanolic extract positive results were obtained for carbohydrate, flavonoid, alkaloid, tannin, glycosides, sterol, proteins & diterpenes. Aqueous extract was also enriched with various phytoconstituents except phenol, tannin, lignin, saponin.

Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture. It can be carried out on an analytical scale to track the development of a reaction or on a preparative scale to purify minute quantities of a chemical. TLC is a popular analytical tool because of its ease of use, relative affordability, high sensitivity, and quick separation.

TLC for flavonoid was carried out for all the four extract. TLC plates were visualized by short UV, long UV & visible light. The Rf value obtained for quercetin was found to be 0.58. Th Rf value obtained for all the four extracts were approximately equal to quercetin, this indicates extract contain quercetin along with other class of flavonoids.

Total flavonoid was found to be highest for ethanolic extract which was 2.44 mg/100mg. The chloroform, ethyl acetate, Aqueous extract estimated to have flavonoid content as 0.77, 1.25 & 1.08 mg/100mg. Also, the alkaloid content was noticed to be highest in ethanolic extract which is 3.14mg/100mg. while, for aqueous extract about 2.31mg/100mg of alkaloid was estimated.

Table 1: Showing the results of percentage loss of *Garcinia indica*

S. No.	Plant Parts	Description	Weight in (gms.)	% loss
1.	Fruits rinds	Weight of plant material in wet, fresh condition	100	**57%**
2.		Weight of plant material after drying at room temperature	43	
3.		Loss in weight on drying	100-43=57	

Table 2: Percentage Yield of *Garcinia indica*

S. No.	Extract	% Yield (w/w)
1.	Chloroform	12.06 %
2.	Ethyl Acetate	1.91 %
3.	Ethanol	12.3 %
4.	Aqueous	2.74 %

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Table 3: Phytochemical Screening of *Garcinia indica*

Sr. No.	Test	Chloroform Extract	Ethyl acetate Extract	Ethanol Extract	Aqueous Extract
1.	Carbohydrate Test Fehlings Test Benedicts Test	 +ve +ve	 +ve +ve	 +ve +ve	 +ve +ve
2.	Phenol Ferric Chloride Test	 -ve	 -ve	 -ve	 -ve
3.	Flavonoid Lead Acetate Test Alkaline Test	 +ve +ve	 +ve +ve	 +ve +ve	 +ve +ve
4.	Alkaloid Wagner's Test	 -ve	 -ve	 +ve	 +ve
5.	Tannin Gelatin Test	 -ve	 -ve	 +ve	 -ve
6.	Lignin Labat Test	 -ve	 -ve	 -ve	 -ve
7.	Saponin Foam Test	 -ve	 -ve	 -ve	 -ve
8.	Glycoside Conc. H_2SO_4 Test	 -ve	 -ve	 +ve	 +ve
9.	Sterols Salkowski Test	 +ve	 +ve	 +ve	 -ve
10.	Proteins Xanthoproteic Test	 -ve	 -ve	 +ve	 +ve

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11.	Diterpenes Copper Acetate Test	+ve	+ve	+ve	+ve

+ve = Positive; -ve = Negative

Table 4: TLC of flavonoids (Quercetin)

S. No.	Mobile phase	*Spot distance*	*Rf*value
1.	Quercetin Dis. travel by mobile phase= 5.5cm No. of spot at long UV= 1 No. of spot at short UV =1 No. of spot at normal light=1	 = 3.2 = 3.2 = 3.2	 = 0.58 = 0.58 = 0.58
2.	Chloroform Extract Dis. Travel by mobile phase=5.5cm No. of spot at long UV =4 No. of spot at short UV =4 No. of spot at normal light= 4	 = 2.6, 3.2, 4.4, 4.9, 5.1 = 3.2, 3.8, 4.3, 4.7, 4.9 = 2.9, 3.5, 4.1, 4.8	 = 0.47, 0.58, 0.80, 0.89, 0.92 = 0.58, 0.69, 0.78, 0.85, 0.89 = 0.52, 0.63, 0.74, 0.87
3.	Ethyl Acetate Extract No. of spot at long UV= 4 No. of spot at short UV = 3 No. of spot at normal light= 2	 = 2.7, 3.1, 3.9, 4.2 = 2.6, 2.8, 4.6 = 3.6, 4.3	 = 0.49, 0.56, 0.70, 0.76 = 0.47, 0.50, 0.83 = 0.65, 0.78
4.	Ethanol Extract No. of spot at long UV= 4 No. of spot at short UV = 4 No. of spot at normal light= 1	 = 2.9, 3.3, 3.6, 4.8 = 2.8, 4.2, 4.6, 5.2 = 4.3	 = 0.52, 0.6, 0.65, 0.87 = 0.50, 0.76, 0.83, 0.94 = 0.78
5.	Aqueous Extract No. of spot at long UV= 0 No. of spot at short UV = 1 No. of spot at normal light= 0	 = 5.2	 = 0.94
6.	1st spot= Quercetin 2nd spot=Chloroform Extract 3rd spot=Ethyl Acetate Extract 4th spot= Ethanol Extract 5th spot= Aqueous Extract		

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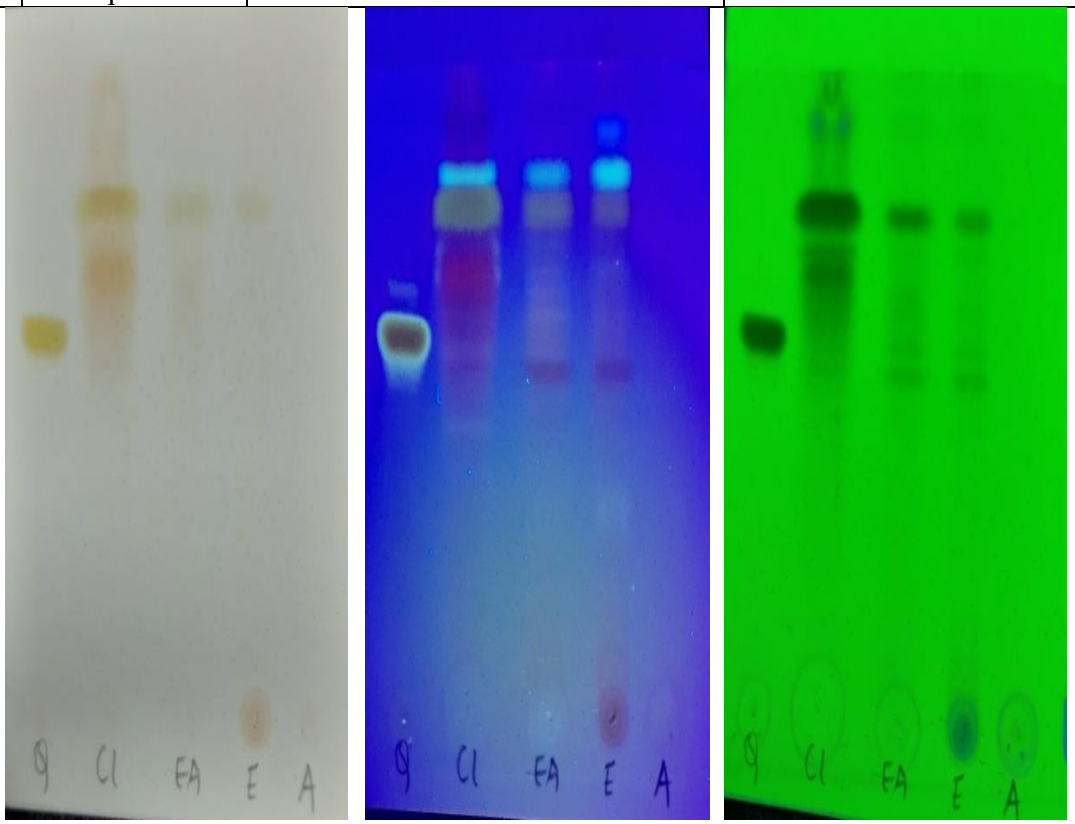
Table 5: Estimation of total flavonoids and alkaloid content of *Garcinia indica*

S. No.	Extracts	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1	Chloroform	0.77	-
2	Ethyl acetate	1.25	-
3	Ethanol	2.44	3.14
4	Aqueous	1.08	2.31



Normal Light **Long UV** **Short UV**

Figure 1: TLC of Flavonoid (Toluene: Ethyl acetate: Formic acid) (5:4:1)

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Conclusion

We discovered that the plant is abundant in phenolic, flavonoid, and alkaloid components in the current investigation, and this has given us some biochemical support for the ethnomedicinal usage of the sample extract from *Garcinia indica*. It has the potential to be a good source of helpful medications as a promising source of bioactive chemicals. Furthermore, it may be deduced that the Ethanolic & aqueous extract of *Garcinia indica* which was previously shown to have antioxidant potential, can work as a considerably more potent anti-oxidant agent.

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