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PHYTOCHEMICAL EVALUATIONS OF *CHUKRASIA TABULARIS* LEAVES

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ABSTRACT

Objective: The aim of the present study was to investigate the presence of phytochemical and to determine the total flavonoid contents in extract of *Chukrasia tabularis*. **Methods:** The plant under evaluation is *Chukrasia tabularis*, a plant of Meliaceae, which is usually found, scattered in evergreen rainforest and deciduous forest. The leaves of the selected plants were removed from the plants and then washed under running tap water to remove dust. The plant samples were then air dried for few days and the leaves were crushed into powder and stored in polythene bags for use. **Results:** The study concluded that the leaf extract have potential bioactive substances that may be used to formulate new and most potent drugs to overcome the problem of disease resistance. **Conclusion:** The total flavonoid content in extract of *Chukrasia tabularis* was found to be 180 mcg respectively.

KEYWORDS: *Chukrasia tabularis*, Meliaceae, Ethyl Acetate, Flavonoids.

INTRODUCTION

Medicinal herbs have been use in one form or another under indigenous systems of medicine. Plant products have been part of phyto medicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, seeds, etc i.e., any part of the plant may contain active components. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers. In the present work, qualitative phytochemical analysis was carried out in *Chukrasia tabularis* plant.

Chukrasia[1,2] is usually distributed in evergreen rainforest, moist semi-evergreen forest and mixed

deciduous forest at altitudes from 20 m to 1450 m. The natural distribution extends from India, Sri Lanka and the east and southeast of southern China to Indochina, Myanmar, Thailand, Peninsular Malaysia (not in the south) and Sarawak, Sumatra (north but rare) and the western tip of Borneo (West Kalimantan, Indonesia). In India, *Chukrasia* occurs in Assam, Arunachal Pradesh, West Bengal, Andaman Islands and in the Indian Peninsula[3,4], mainly on the Western Ghats.[5] It also extends to Konkan, in North Kanara (Karnataka), the Sandur Hills in Bellary, in Salem (Tamil Nadu) and in Orissa. (**Fig. 1) (Fig. 2).**

Fig.1:*Chukrasia tabularis* Stem and Branches

Fig. 2:*Chukrasia tabularis* Flowers and Leaves

From the literature review plant was reported to be rich in Limonoids[6,7,8,9], Phragmalin derivatives[10], Tannins, Flavonoids and other Phenolic Compounds. The various chemical constituents isolated so far are Sitosterol, Quercetin, 7-Dimethoxycoumarin, Scopoletin, Cedrelone, Tannic Acid, Tabulalin, Tabulalide A, Tabulalide B, Tabulalide C, Tabulalide D, Tabulalide E, Melianone, Bussein Homologue, Chukrasin A-E, Tabularin, 12-acetoxyphragmalin 3,30-diisobutyrates, 12-Acetoxy phragmalin3, isobutyrates-30-Propionates, Phragmalin3, 30 Diisobutyrates, Phragmalin3,Isobutyrates-30-Propionates, Chuktabrin A-B, Chuktabularin A-D, Tabularisin A-P, (24R)-28,29-dinorcycloartane-3,24,25-Triol, Chukvelutilide A- F.

MATERIALS AND METHODS

Collection and Authentication

Young leaves of *Chukrasia tabularis* A. Juss were collected from Dharavarithota, Ongole. The plant was authenticated by Dept. of Botany, Hindu College, Guntur.

Preparation of Plant Extract

The leaves of the selected plants were removed from the plants and then washed under running tap water to remove dust. The plant samples were then air dried for few days and the leaves were

crushed into powder and stored in polythene bags for use.

Extraction by successive solvent extraction

The coarse powdered leaves of the *Chukrasia tabularis* were initially extracted by Successive solvent extraction[11] by using soxhlet apparatus.[12] The extraction was carried by using different solvents in increasing polarity with Petroleum Ether (BP 60-80˚c), Chloroform, Ethyl acetate, Methanol and water. The contents of the flask were collected separately after each solvent and were concentrated on Heidolph Rotavac under vacuum. The % yield of Successive solvent extracts was shown in **Table 1**.

Table 1: **% yields of Successive solvent extracts of *Chukrasia tabularis***

S. No	Extract	% yield
1	Pet. ether	2.4
2	Chloroform	2.1
3	Ethyl acetate	3.4
4	Methanol	44.77
5	Water	5.67

The obtained extracts were then subjected to preliminary phytochemical evaluation to know the compounds present in the extracts and results are shown in **Table 2**.

QUALITATIVE EVALUATION OF PHYTOCHEMICAL

Test for carbohydrates[13]

Fehling's test

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's test

Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Test for proteins[14]

Millon's test

Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Ninhydrin test

Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

Test for steroid[15]

Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Test for flavonoids

Shinoda test

Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of Flavonoids.

Alkaline reagent test

Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of Flavonoids.

Test for alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Test for glycosides

Liebermann's test

Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Salkowski's test

Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

Test for phenols and tannins[16]

Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Table 2: **Phytochemical Screening of Successive Solvent Extracts**

Compounds	Pet. ether	Chloroform	Ethyl acetate	Methanol	Water
Carbohydrates	-	+	+ +	+ + +	+ + +
Proteins	-	-	-	-	-
Fats & oils	+ + +	+ +	+ +	+	-
Steroids	-	+	-	+ +	-
Flavonoids	-	-	+ + +	+ +	-
Alkaloids	-	-	-	-	-
Glycosides	-	+	+	+	+
Tannins & phenolic	-	+	+	+	+

(Note: + = indicates presence of phytochemical and

- = indicates absence of phytochemical.

++ = shows moderate concentration.

+++ = shows high concentration.**)**

Solvent- solvent separations

The methanolic extract was taken into a beaker and was dissolved in distilled water. The obtained aqueous methanolic extract was then transferred into a separating flask. To this solvents were added in increasing order of polarity. Initially, Hexane was added to the aqueous methanolic extract. The separatory flask was then shaken vigorously and was allowed to stand still; the immiscible liquids were then allowed to separate out completely. Later the liquids were collected separately. The process was repeated thrice with the same solvent and the hexane fractions are pooled together. The above process was repeated using different solvents in increasing order of polarities i.e., Chloroform, Ethyl acetate and n-Butanol were added sequentially. Each fraction was collected and concentrated on Heidolph Rotavac[17] under vacuum. The solvent-solvent separation technique helps in easily and effectively separating the obtained methanol extract was shown in **Table 3**.

Table 3: % yield of various fractions obtained from Solvent- Solvent separations

S. No	Fraction	% yield
1	Hexane	1.72%
2	Chloroform	0.69%
3	Ethyl acetate	19.69%
4	n-Butanol	1.93%

Ethyl acetate fraction was found to be 19.69%. All the fractions were subjected to phytochemical screening for identifying various phyto-constituents and results were shown in **Table 4**.

Table 4: **Phyto chemical screening of fractions obtained from methanol extract**

Compounds	Hexane	Chloroform	Ethyl acetate	n-Butanol
Carbohydrates	-	+	+ +	+ + +
Proteins	-	-	-	
Fats & oils	+ + +	+ +	-	-
Steroids	-	+	-	-
Flavonoids	-	-	+ + +	+
Alkaloids	-	-	-	-
Tannins & phenolic	-	+	+	+
Glycosides	-	+	+	-

(Note: + = indicates presence of phytochemical and

- = indicates absence of phytochemical.

++ = shows moderate concentration.

+++ = shows high concentration.**)**

Phytochemical screening of Methanol extract revealed the presence of Carbohydrates, Flavonoids, Steroids and Tannins & Phenolic Compounds. The obtained methanolic extract was then subjected to Solvent- Solvent Separations Method.

Column chromatography

Pen column was taken for performing column chromatography.[18] The column length is 30 cm and diameter is 0.5 cm using Silica gel 100-20 mesh as adsorbent and Hexane, Ethyl acetate, Methanol, water as Eluent in gradient technique.[19,20] The resultant extract was subjected to column chromatography. The obtained ethyl acetate extract was passed through it isolation of chemical constituents. The isolated compounds were shown in **Table 5.**

Table 5: Phytochemical screening of fraction's obtained by Column Chromatography

Tests	F3	F4	F5	F6	F10	F35	F36
Carbohydrates	-	-	-	-	-	+	+
Proteins	-	-	-	-	-	-	-
Steroids	-	-	-	-	-	-	-
Glycosides	+	+	+	-	-	-	-
Flavonoids	-	-	+	+	+	-	-
Tannins & Phenolic	+	+	+	+	-	+	-

(Note: + = indicates presence of phytochemical and

- = indicates absence of phytochemical**.)**

Spectral analysis of F10

Spectral analysis is being carried out in order to find out the exact components present in the plant and to characterization of active constituents in the compound by spectral analysis. The spectral analysis includes the usage of IR, H^1 NMR and Mass Spectrum respectively. From the spectral analysis of F10 and phytochemical screening[21], it was assumed that the compound F10 contains a Flavonoid nucleolus. **(Fig. 3) (Fig. 4) (Fig. 5) (Fig. 6)**

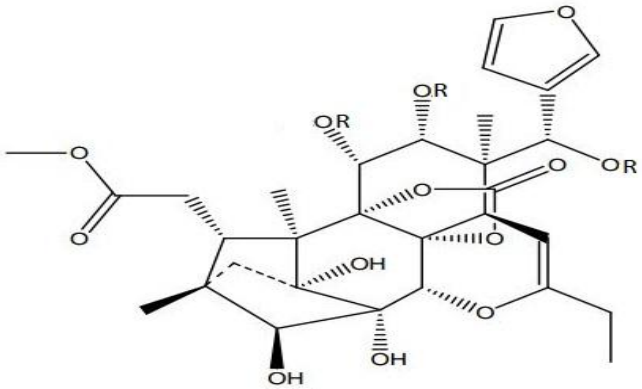

Fig. 3: Possible structure of identified Flavonoid

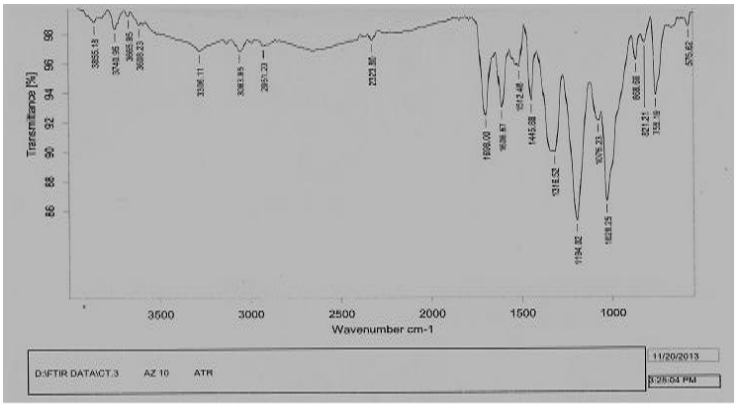

Fig. 4: IR spectrum of F10 fraction

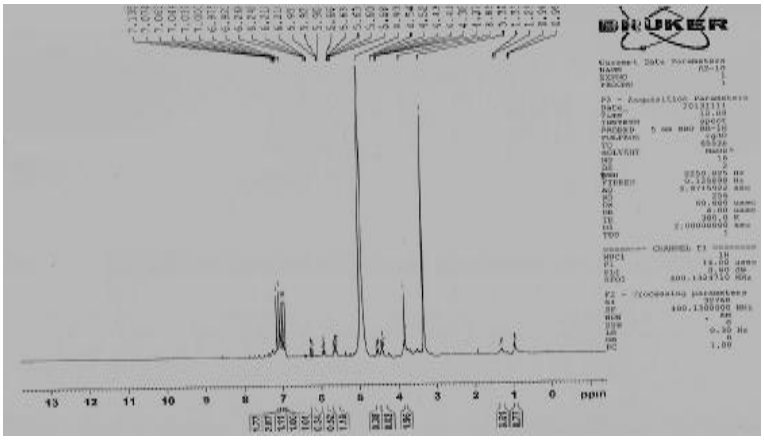

Fig. 5: 1H NMR of F10 fraction

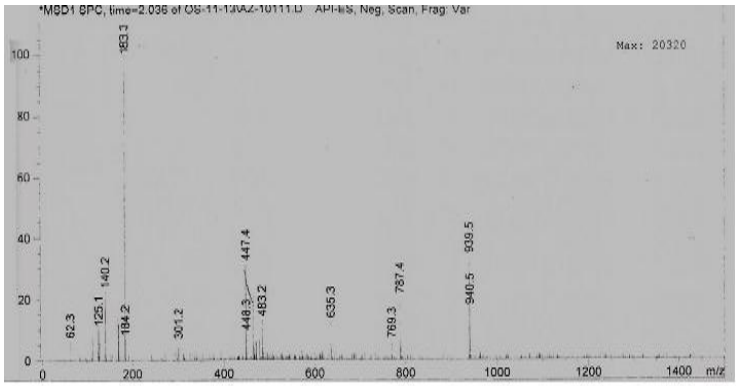

Fig. 6: Mass spectrum of F10 fraction

QUANTITATIVE EVALUATION FOR TOTAL FLAVONOID CONTENT

The Ethyl acetate fraction of the methanolic extract was estimated for total flavonoid content[22] in it. The mixture consisting of 1ml of extract solution, 0.3ml of 5% sodium nitrite and 0.3ml of 10% aluminum chloride was incubated for 5 minutes, followed by adding 2ml of 1M sodium hydroxide solution. The volume of the mixture was raised to 10ml and it was then thoroughly vortexed. The absorbance of pink colored solution was determined at 510nm.

Test solution

0.5 mg/ml of the methanol extract solution was prepared for estimating the total flavonoid content.

Standard solution

100 mg of the methanolic extract was accurately weighed and transferred to 10 ml volumetric flask and made up the volume with methanol.

Calibration curve method

The calibration curve was plotted by 20 - 100 mcg/ml concentrations of standard flavonoid solutions. 0.5 mg/ml concentration of test solution was used for estimating the total flavonoid content[23,24] in the Ethyl acetate fraction. From the calibration curve, 1 mg of dried Ethyl acetate fraction was estimated to contain around 180 mcg of Flavonoids. The value obtained was mean of 6 tests. **(Fig. 7).**

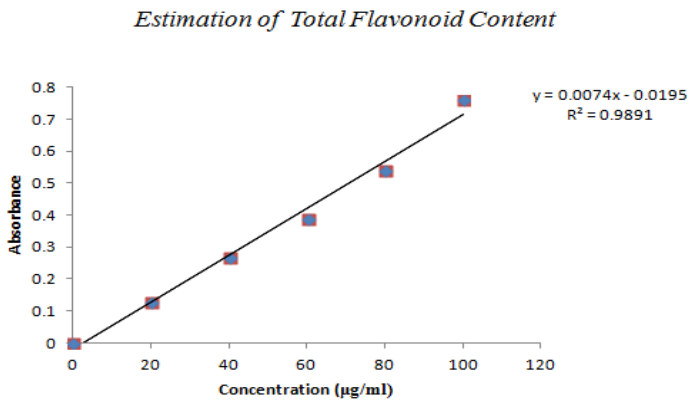

Fig. 7: Calibration Curve for the estimation total flavonoid content

CONCLUSION

The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. Exploitation of these pharmacological properties involves further investigation of these active ingredients by implementation techniques of extraction, purification, separation, crystallization and identification. The *Chukrasia tabularis* A. Juss leaves were extracted, separated and isolated. The methanol extract was first prepared and was screened for various phytochemical constituents. From this methanol extract Ethyl acetate fraction was obtained using Solvent- Solvent separation technique. The fraction was screened for spectral studies of phyto-constituents. The Ethyl acetate fraction of the methanol extract was found to be 180 mcg of Flavonoids. Furthermore evaluation is needed to isolate the bioactive substances which can be used for welfare of the mankind.

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