

## Bioassay Guided Fractioning of Active Principle from *Cassia Alata.L*

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**Abstract:** *Cassia alata* having the effective fungicidal properties. It is used for treating ringworm and other fungal infection of the skin. So it is termed as ringworm bush. *Cassia alata* having the properties of antifungal and antibacterial. It is a common ingredients in soaps, shampoo and lotion. In the current study, secondary metabolites were extracted from *Cassia alata* by using different solvents like hexane, chloroform, ethanol to detect the presence of bioactive compounds. Qualitative analysis was done for various constituents like tannins, carbohydrates, saponins, quinines, flavonoids, alkaloids, glycosides, cardiac glycosides, terpenoides, triterpenoides, phenols, coumarins, phytosteroids and steroids, phlobatannins and anthraquinones. Quantitative analysis of Tannins and Flavonoids was estimated. From plant sources we derived many of the antioxidant compounds which is present in a typical diet. These compounds are belongs to various classes with a huge variety of physical and chemical properties. The antioxidant potentials were determined by DPPH and FRAP assay and the result showed that the high activity was present only in ethanol extract than others. The various antioxidant assay conclude that extract of ethanol has high activity of compounds. Hence and it was further proceed by column chromatography and thin layer chromatography. The overall results revealed that the quercetin bioactive compounds had high antioxidant activity and it is used for curing diabetes, cataracts, hay fever, viral infections etc.

**Keywords:** Antioxidant activity, *Cassia alata*, Column chromatography, Phytochemical screening, TLC.

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### I. Introduction

Many tropical countries and in India. *Cassia* species (Caesalpinaceae) are an important medicinal plant. This plant have great a potential in traditional medicine and pharmacological drugs. A large portion of the world most of the people depends on traditional medicine for many diseases. The scarcity and high costs of orthodox medicine many of them followed natural medicine [1]. The plant grows in ditches and rice-fields. The plant propagation carried by seeds. Distribution around all over the country. The plant mainly cultivated for medicinal purposes [2].

*Cassia* species contain polyphenol compounds such as steroids, anthraquinones, flavonoids, and which exhibited strong antioxidant activity [3], [4]. In traditional medicine *Cassia* species are well known for skin disease treatment because *Cassia* having purgative and laxative properties[5]. *Cassia* species having many secondary metabolites like glycosides, alkaloids, flavonoids, tannins, steroids, terpenoids, essential oils and phenolic compounds [6], [7], [8]. Thin layer chromatography used to monitor the progress of a reaction. It can be used to identify the presence of compounds and also purity of a given mixture. In the present study was under taken to look for phytochemicals. The antioxidant assay of *Cassia alata* leaf extracts using DPPH and FRAP assay.

### II. Materials and Methods

#### 2.1 Sample Collection

The *Cassia alata.L*, leaves were collected from guindy, Chennai.

#### 2.2 Sample Extraction

*Cassia alata .L*, were collected, washed with tap water and then rinsed in the distilled water finally the cleaned leaves are air dried. The dried leaf of each plant was pulverized using a sterile electric blender, to make a fine powder and stored. For the preparation of aqueous extract of the plant samples were soaking 100gms of dry powdered samples in 1:3 of ratio in various solvents : a) hexane(1:3) b) chloroform(1:5) c) ethanol (1:2) 12 hours. The extracts were filtered using Whatmann filter paper No. 42(125mm). The filtrate were stored at room temperature in a airtight dark bottles. Dried plant material was used as a source for the extraction of bioactive compounds in plants [9].

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### 2.3 Qualitative Phytochemical Analysis

The various qualitative chemical tests were carried out by using standard procedures described by Sofawara (1993) [10].

### 2.4 Quantitative Phytochemical Analysis

#### 2.4.1 Flavonoids Determination

Each plant extract (1mg/1ml) were prepared and 100µl of each sample was taken in separate tubes and made up to 1ml with 2.8 ml methanol. 0.1ml of 10% aluminium chloride and 0.1ml of 1M potassium acetate was added and kept at room temperature for 30minutes. The absorbance in mixture was measured at 415 nm.

#### 2.4.2 Determination of Tannins

The extracts were aliquoted in required concentrations (100µl of sample dissolved in 900µl of water) and made up to 1 ml with distilled water and then mixed with 0.5 ml of Folin-Ciocalteu reagent. The mixture was alkalized by the addition of 1ml of 15 % ( w/v) sodium carbonate solution and kept at room temperature in dark for 30 minutes. By using spectrophotometer the absorbance of the solution was read at 700nm, and the pure tannic acid used as standard for determine the concentration of tannins.

### 2.5 Antioxidant Free Radical Scavenging Activity

#### 2.5.1 DPPH

The 2,2 -Diphenyl -1-picrylhydrazyl (DPPH) free radical scavenging activity was performed as described by Yamasaki *et al* .,1994 [11].

#### 2.5.2 Frap Assay

The FRAP assay uses antioxidants as reductants in a redox- linked colorimetric method employing an easily reduced oxidant colourless ferric to blue colour ferrous can be monitor by measuring absorbance. 1mg/ml of sample and make upto 1ml with distilled water and mix with 1.5ml of working frap solution and incubated at 37°C for 4minutes .The absorbance of the sample measured at 593nm .

### 2.6 Chromatography

#### 2.6.1 Column Chromatography

Take 20g of silica gel and 6g grams of ethanol extracts mixed with hexane solvents and pour into the column and separate compounds in fraction wise and then identify particular compound in the thin layer chromatography plate [12].

#### 2.6.2 Thin Layer Chromatography

Solvent system toluene, ethyl acetate, and formic acid (4:5:1). To analyze the pure compounds on TLC studies were carried out by following the methods of (khan 2001) [13].

## III. Results and Discussion:

Phytochemical screening is playing an important role in the scientific assessment of the therapeutic potency. The phytochemicals have the healing potency which taken a new dimension and the Scientists are interested in constitutes each medicinal plants. *Senna alata* are reported to contain many variety of secondary or bioactive compounds, known as phytochemicals. Now a days the identification and isolation of medicinally important new therapeutic compounds are derived from higher plants for specific diseases [14]. Majority of the people in world using herbal remedies for diseases [15]. Herbal plants having many pharmacologically important bioactive compounds like tannin, steroids, glycosides, flavonoids, alkaloids, phenols, fixed oils, which is stored in their specific parts of leaves, bark, flowers, seed, fruits, root etc [16].

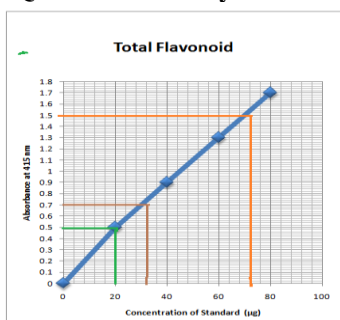
In the present study the preliminary phytochemical screening of *C.alata* leaves done with three different extracts hexane, chloroform and ethanol. Flavonoids, steroid and phytosteroids are present in all three extracts. Flavonoids ( $1.8 \pm 0.03 \mu\text{g/g}$ ), tannin ( $1.2 \pm 0.02 \mu\text{g/g}$ ) is estimated in ethanol extract by quantitative analysis.

**3.1 Qualitative Analysis of Phytochemicals**

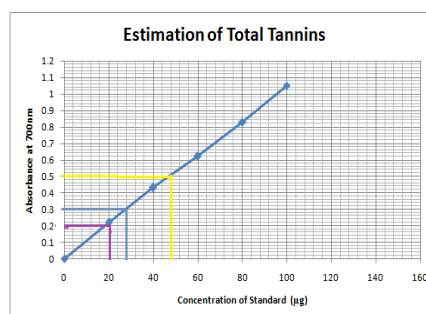
**TABLE:1 Phytochemical analysis of *Cassia alata*.1**

Phytochemicals	Hexane	Chloroform	Ethanol
Carbohydrates	Absent	Slightly present	Present
Tannins	Present	Present	Absent
Saponins	Absent	Absent	Slightly present
Flavonoids	Present	Present	Present
Alkaloids	Present	Present	Slightly present
Quinones	Absent	Absent	Absent
Glycosides	Absent	Present	Absent
Cardiac Glycosides	Present	Absent	Present
Terpenoids	Present	Absent	Present
Triterpenoids	Absent	Absent	Absent
Phenols	Slightly present	Present	Present
Coumarins	Present	Present	Present
Steroid and phytosteroids	Present	Present	Presence
Phlobatannins	Absent	Absent	Absent
Anthraquinones	Absent	Absent	Absent

**3.2 Quantitative Analysis**



**Fig:1 Flavonoids Estimation:**



**Fig:2 Tannins estimation**

The total phenolic contents and antioxidant activities of aqueous and ethanolic extract of *Senna alata*. The antioxidant activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods. The strongest antioxidant activities of aqueous extract of

Senna alata were  $22.11 \pm 0.324$  mg gallic/g extract and  $214.99 \pm 17.279$  mg trolox/g extract when determined by DPPH and ABTS assay, respectively. The aqueous extract showed the highest total phenolic content of  $70.90 \pm 1.048$  mg Gallic/g extract [17].

Phenolic compounds and flavonoids have been reported to be associated with antioxidant action in biological systems, mainly due to their presence of redox properties. These properties can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Flavonoid having their excellent radical scavenging ability. Total phenolic contents were determined and amounted to  $74.35 \pm 0.89$ . The contents of flavonoids were determined and amounted to  $24.37 \pm 0.25$  [18].

Phenolic compounds, act as a natural antioxidants. The plants having phenolic compounds such as flavonoids, phenolic acids, tocopherols etc [19]. Quercetin, abundant dietary flavonol, which is used as a potent antioxidant due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, inhibition of enzymes responsible for free radical generation [20].

In this study agreed with the above results the higher antioxidant activity in ethanol extract i.e  $53.66 \pm 0.89$  mg gallic/g elucidated on DPPH assay. FRAP Value = (Change in absorbance of sample from 0 to 4 mins/Change in absorbance of standard from 0 to 4 mins  $\times$  FRAP value of standard. Absorbance changes indicates the presence of antioxidant in mixture. The sample FRAP value was calculated and it was found to be 1.64 for ethanol extract and 1.62 for chloroform extract. FRAP value of sample and standard were comparable. Ethanol extract showed the higher antioxidant activity .

### 3.3 Antioxidant Assay

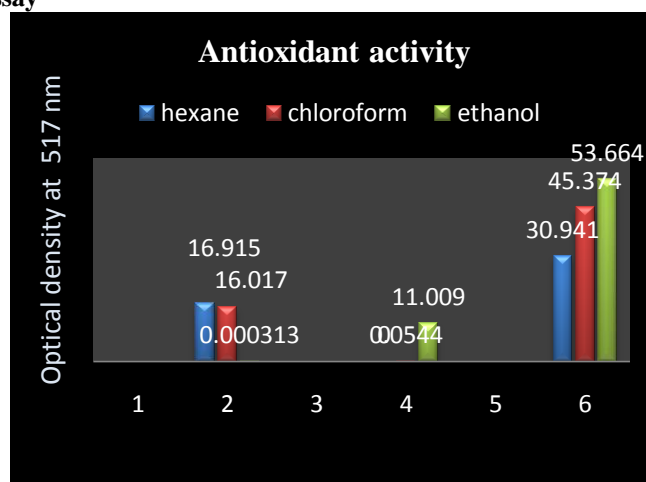


Fig: 3 DPPH Assay

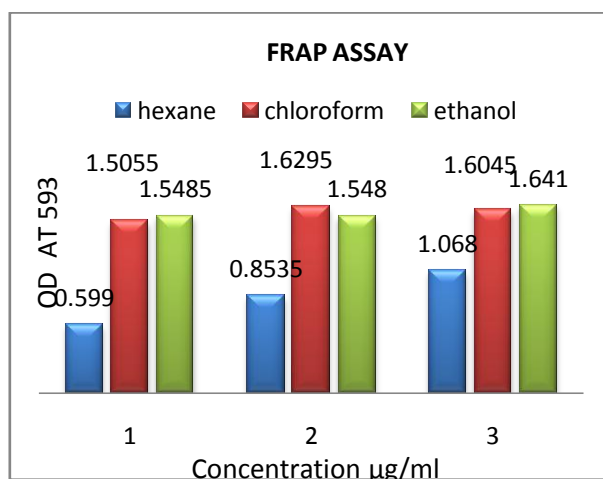


Fig: 4 FRAP Assay

Qualitative detection of antioxidant compounds in *C. alata* extract revealed compounds with Rf values of 0.25, 0.27, and 0.25 extracted with ethanol, methanol, and acetic acid, respectively, the first of which represented gallic acid (Rf = 0.24) where the other compound found at Rf 0.41, 0.45, and 0.56 of ethanol, methanol, and acetic acid, respectively, have not been identified [21].

*Sesbania sesban* having quercetin compound in methanolic stem extract which is a sharp and well-defined yellow colour band with Rf = 0.71 for quercetin. According to Mythili et al., the quercetin bioactive compound may be responsible for the activity of astringent, anti-inflammatory and carminative purgative of *Sesbania sesban* [22].

In this study *Cassia alata* leaf extract of ethanol was fractioning by column and Thin Layer Chromatography. Column chromatography gave five fractions which were collected based on colour difference. Each fraction was performed on thin layer chromatography and identifies the pure compounds in the *Cassia alata* leaves. To analysis from the extract, the band was obtained with the RF values 0.80, 0.82, 0.84, respectively. Each of the fractions was subjected to antioxidant activity to finding the bioactive compound in the *Cassia alata* leaf. In the result conclude that the highest activity of antioxidant in the *Cassia alata* due to the presence of quercetin compound.

### 3.4 Thin Layer Chromatography

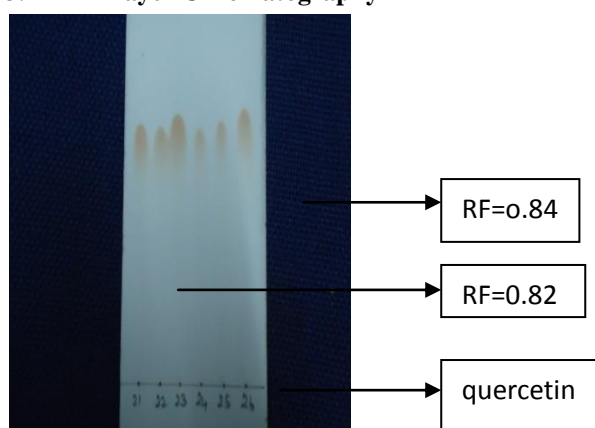


Fig: 5 spraying reagent

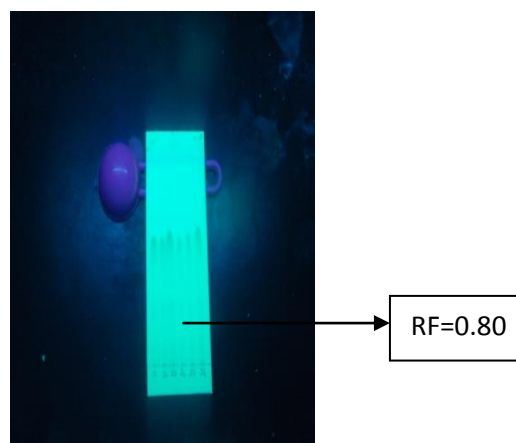


Fig: 6 ultraviolet lamp

## IV. Conclusion

Scientists are interested to finding new antioxidant sources which having the remarkable health benefits. Discovering a natural source of antioxidants could also be significant for artificial toxic antioxidants replacement in food industry. The results of this study clearly indicated that ethanolic leaf extract of *Cassia alata* are good scavengers of synthetic DPPH radicals, indicating that they could be used as antioxidant products. The overall results revealed that the quercetin bioactive compounds had high antioxidant activity and it can be used for curing diabetes, cataracts, hay fever, viral infections etc.

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