

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.608.056>

## Extraction of Bio Active Compounds from *Cassia Auriculata* Pods and Leaves and its Medicinal Uses

M. Monisha<sup>1</sup>, M. Sowmiya<sup>1</sup>, R. Ragunathan<sup>2\*</sup> and JESTEENA JOHNEY<sup>2</sup>

<sup>1</sup>Department of Biotechnology, Bannari Amman Institute of Technology,  
Sathyamangalam-401, Tamil Nadu, India

<sup>2</sup>Centre for Bioscience and Nanoscience Research, Eachanari, Coimbatore –21,  
Tamil Nadu, India

\*Corresponding author

### ABSTRACT

#### Keywords

*Cassia auriculata*,  
Phytochemical  
studies, TLC,  
Antimicrobial  
activity, Anti-  
inflammatory  
activity.

#### Article Info

Accepted:  
04 June 2017  
Available Online:  
10 August 2017

*Cassia auriculata* used for long period in various chronic diseases therapeutically. The present work attempts to investigate phytochemical studies of leaves and pods of *Cassia auriculata*. Hence it have antimicrobial, antioxidant, anti-inflammatory properties and the present study of aqueous and methanol extracts were used to identify the medicinal properties of *Cassia auriculata*. With reference to the above claims, the results of phytochemical, antioxidant, antimicrobial, anti-inflammatory, chromatography studies of leaves and pods have been described.

### Introduction

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. In rural areas of the developing countries they continue to be used as the primary source of medicine (Chitme *et al.*, 2003). About 80% of the people in developing countries use traditional medicines for their healthcare (Kim *et al.*, 2005).

The natural products derived from medicinal plants have proven to be an abundant source

of biologically active compounds, many of which have been the basis for the development of new lead chemicals for pharmaceuticals. As there are approximately 5, 00,000 plant species occurring worldwide, of which only 1% has been phytochemically investigated, there is great potential for discovering novel bioactive compounds.

#### Plant description

*Cassia auriculata* Linn, family  
Caesalpinaceae, is also known as

*Senna auriculata*. The leaves are alternate, stipulate, very numerous, closely placed, rachis 8.8-12.5 cm long, narrowly furrowed, slender, pubescent, with an erect linear gland between the leaflets of each pair, leaflets 16-24, very shortly stalked 2-2.5 cm long 1-1.3 cm broad, slightly overlapping, oval oblong, obtuse, at both ends, glabrous or minutely downy, dull green, paler beneath, stipules very large, produced at base on side of next petiole into a fill form point and persistent. Its flowers are irregular, bisexual, bright yellow and large (nearly 5 cm across), the pedicels glabrous and 2.5 cm long. The racemes are few-flowered, short, erect, and crowded in axils of upper leaves so as to form a large terminal inflorescence (leaves except stipules are suppressed at the upper nodes). The 5 sepals are distinct, imbricate, glabrous, concave, membranous and unequal, with the two outer ones much larger than the inner ones (Joy *et al.*, 2012).

## **Materials and Methods**

### **Collection of plant materials**

The plant material was collected from the botanical garden of CBNR (Centre for Bioscience and Nano science Research). The leaves and pods were dried under sunlight for 2-3 days and then milled into powder form which was used to prepare extracts.

### **Preparation of plant extract**

#### **Aqueous extract**

One gram(1g) of dried powder of experimental material of both the leaves and pods were soaked in 20 mL of water for 24hrs and kept in shaking incubator at 50-60 rpm & 40°C. The mixture was then filtered through the Whatmann No.1 filter paper to ensure that no particles were present in the solution and the extract was collected.

#### **Organic extract (Methanol)**

One gram (1g) of powdered material of both the leaves and pods were soaked in 20 mL of methanol and kept at shaking incubator at 50-60 rpm & 40°C for 24 hrs. The mixture was then filtered with Whatmann No.1 filter paper and the extract was collected.

### **Phytochemical screening**

Each dry extract was used for screening the following bioactive compounds: alkaloids, terpenoids, phenol and tannins, sugar, saponins, flavonoids, quinines, and steroids and proteins, according to the method described by Ayoola *et al.*, (2008).

#### **Alkaloids test**

1ml of extract was mixed with 1ml of Mayer's reagent and few drops of iodine solution also added, the formation of yellow colour indicates the presence of alkaloids.

#### **Terpenoids test**

1ml of extract was mixed with 1ml of Conc.H<sub>2</sub>SO<sub>4</sub> and incubated in water bath for 2-4 minutes, the formation of greyish colour indicates the presence of terpenoids.

#### **Phenol and tannins**

1ml of extract was added to 1ml of 2% FeCl<sub>3</sub>. The formation of blue, green or black colour indicates the presence of tannins.

#### **Test for sugar**

One ml of Fehling's A solution and 1ml of Fehling's B solution was mixed with 1ml of extract and incubated in water bath for 2-4 minutes, the formation of red colour settled in the bottom of the tube indicates the presence of sugar.

### **Test for saponins**

1ml of extract was mixed with 1 to 2ml of distilled water, the formation of 1cm foam layer indicates the presence of saponins.

### **Test for flavonoids**

The few fragments of magnesium ribbon was added to 1ml of extract and then add few drops of Conc. HCl. The formation of pink scarlet colour indicates the presence of flavonoids.

### **Test for quinones**

1ml of extract was mixed with 1ml of 2% NaOH. The formation of blue green or red indicates the presence of quinones.

### **Test for protein**

Few drop of Conc. nitric acid was added to 1ml of extract, the formation of yellow colour indicates the presence of proteins.

### **Test for steroids**

1ml of extract was added to 1ml of chloroform. After 2-3 minutes 1ml Conc.H<sub>2</sub>SO<sub>4</sub> was added. A red colour produced at the lower chloroform layer indicates the presences of steroids.

### **Antioxidant activity**

#### **DPPH -1, 1-diphenyl-2-picryl-hydrazyl Activity**

1ml of sample and 0.1ml of 1M DPPH Solution were incubated at room temperature for 5 minutes and then added 0.4ml of 50 mM TrisHCl. These solution mixtures were incubated again in room temperature and in dark condition for 30 minutes. Then OD value was measured at 517nm using

spectrophotometer, mg/g of DPPH was calculated with standard ascorbic acid.

### **Test for flavonoids**

1ml of sample was added to 0.1ml of 10% aluminium chloride. After few minutes 0.1ml of 1M Potassium acetate was mixed. Then 2.8ml of distilled water was added and incubated at room temperature for 30 minutes. Finally OD value was taken at 415nm and mg/g of flavonoids were calculated by using Quercetine as a standard.

### **Test for phenols**

0.5 ml of samples was taken and 0.5ml of Folin Ciocalteu was added. After few minutes 1ml of 20% sodium carbonate was added and incubated at 45°C for 15 minutes. Then the OD value was observed at 765nm using spectrophotometer. Gallic acid is used as a standard to calculate the amount of phenol content in mg/g (Nisha *et al.*, 2016).

### **Antimicrobial activity**

#### **Antibacterial activity**

For the antibacterial study, cultures of *Staphylococcus aureus* (gram positive), *Escherichia coli* (gram negative), and *Pseudomonas aeruginosa* (gram negative) were used. The bacterial stock cultures were stocked at 4°C. Mueller Hinton Agar were prepared and sterilized at 121°C for 15minutes. The antibacterial assays were carried out by the well-diffusion method (Jesteeena *et al.*, 2016).

#### **Well diffusion method**

Antibacterial screening was studied by using well diffusion method. Mueller Hinton agar plate were prepared and after solidification 60 µl of *Staphylococcus aureus*, *Escherichia coli*

and *Pseudomonas aeruginosa* were poured and spread with sterile cotton swab and kept for drying for 2-3 minutes. Wells were made with cork borer in the diameter of 5mm and added methanol, water plant extract 1ml is poured in each well. DMSO was used as negative control and antibiotic disc Erythromycin used as a positive control. After plating all the plates were incubated at 37°C for 24 hours. The diameters of inhibition zone produced by the plant extract were measured in mm.

### **Antifungal activity**

Standard fungal cultures *Aspergillus niger* and *Trichoderma viride* were used. The fungal stock cultures were maintained on potato dextrose agar. Antifungal screening is performed by the above explained well diffusion method (Jestena *et al.*, 2016).

### **Anti-inflammatory activity**

### **Anti-proteinase action**

1ml of sample was added to 0.06mg of trypsin and 1ml of 20mM trisHCl. These solution mixtures were incubated at 37°C for 10-15 minutes. After few minutes 0.8% casein was mixed and incubated at room temperature for 20-30 minutes. And 1.5ml of 7% perchloric acid was added to arrest the reaction. The suspension was centrifuged at 5000rpm for 5mins, pellet was discarded and the supernatant were used to read the OD value at 210 nm (Akah *et al.*, 2003).

### **Chromatographic techniques**

Chromatography is a technique for the separation of a mixture by passing it in solution or suspension through the medium in which the components move at different rates. It has two phases, one stationary and the other mobile phase moving in a definite direction. Here bioactive compounds were analyzed by

paper chromatography and Thin Layer Chromatography (TLC) (Homans and Fuchs, 1970).

### **Paper chromatography**

In this technique, the mixture containing the pigments to be separated is first applied as a spot or a line to the paper about 1cm from the bottom edge of the paper. The paper is then placed in the container with the tip of the paper touching the solvent.

The solvent is absorbed by the paper and moves up the paper by capillary action.

As the solvent crosses the area containing plant pigment extract, the pigments dissolve in and move with the solvent. The pigments are carried along at different rates because they are not equally soluble. Therefore, the less soluble pigments will move slower up the paper than the more soluble pigments.

### **Thin Layer Chromatography (TLC)**

A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid absorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase and it slowly rises up the TLC plate by capillary action.

When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried and the separated components of the mixture are visualized. If the compounds are coloured, visualization is straight forward. Usually the compounds are not coloured, so a UV lamp is used to visualize the plate.

## Results and Discussion

### Phytochemical screening of *Cassia auriculata*

Phytochemical analysis of *Cassia auriculata* reveals the presence of alkaloids, flavonoids, phenols, tannins, saponins in maximum amount. Methanol extracts of pod showed good results than the Aqueous extracts of leaves in analyzing the Bio activecompounds and they are shown in table 1.

### Antioxidant activity

DPPH 1, 1- Diphenyl- 2- picryl- hydrazyl (DPPH) activity were calculated in mg/g with the standard ascorbic acid. 14 mg /g of methanol and 3 mg/g of water were present in the *Cassia auriculata*. Hence methanol extract of pod shows more antioxidant activity than the water extract of leaves (Aurelia *et al.*, 2011). The mg/g of DPPH present in sample is given in table 2.

### Test for phenols

Total phenolic content of methanol and water extracts were calculated in mg /g with the standard gallic acid.148 mg/g of phenolic content is present in Methanol extract and 148 mg/g of total phenol is present in water extract of *Cassia auriculata*. Here both the methanol extract and the water extract contains the same level of phenol content (Table 3).

### Test for flavonoids

The Total flavonoid content of *Cassia auriculata* was calculated in mg/g with Quercetine as the standard. The total flavonoid content calculated was 283 mg/g in methanol extract and 14 mg/g in water extract. Hence Methanol extract shows more flavonoid content in *Cassia auriculata* than the water extrat of leaves (Table 4).

## Antimicrobial activity

### Antibacterial activity

The results of antibacterial activity of the aqueous and organic leaf and pod extracts of *Cassia auriculata* against *E. coli*, *P. aeruginosa* and *S. aureus* are reported in table 5. Methanol extract showed more antibacterial activity with the three bacteria with inhibitory zones of 5 mm, 10 mm and 3mm respectively for *E. coli*, *P. aeruginosa* and *S. aureus*. The aqueous extracts second in efficacy showed mean inhibitory zones of 5mm, 8 mm and 2 mm respectively for *E. coli*, *P. aeruginosa* and *S. aureus*. There was no inhibition zone for DMSO, whereas disc had the widest inhibitory zones of 5 mm, 10 mm and 6 mm respectively for *E. coli*, *P. aeruginosa* and *S. aureus*.

Results of the agar-well diffusion test showed that the methanol fruit extract of *Ziziphusspina-christi* has varied degrees of antibacterial effect in *Staphylococcus epidermidis* as  $11.0 \pm 1.0$  and  $10.5 \pm 0.5$  mm, followed by *Staphylococcus aureus* as  $11.0 \pm 0.0$  and  $7.7 \pm 0.7$  mm and *Bacillus cereus*  $10.5 \pm 0.5$  and  $8.2 \pm 0.7$  mm respectively(Abdallah,2017).

### Antifungal activity

The results of antifungal activity of the aqueous and organic leaf and pod extracts of *Cassia auriculata* against *Aspergillus niger* and *Trichoderma viride* are reported in table 6. Here also Methanol extract showed more antifungal activity with inhibitory zones of 2 mm and 2 mm respectively for *Aspergillusniger* and *Trichoderma viride*.

The aqueous extracts second in efficacy showed mean inhibitory zones of 1 mm and 1 mm respectively for *Aspergillus niger* and *Trichoderma viride*. There was no inhibition zone for DMSO.

**Table.1** Phytochemical constituents of *Cassia auriculata*

Phytochemical Tests	Leaves		Pods	
	Methanol	Water	Methanol	Water
Alkaloids	+	+	+	+
Terpenoids	-	+	-	+
Phenols and tannins	+	+	+	+
Sugar	-	+	+	-
Saponins	+	+	-	+
Flavonoids	+	+	+	+
Quinones	+	-	+	-
Proteins	-	+	+	-
Steroids	-	-	-	+

(+): presence of chemical constituents, (-): absence of chemical constituents

**Table.2** Antioxidant- DPPH activity of *Cassia auriculata*

Sample	DPPH activity(mg/g)
Pod (methanol)	14
Leaves(water)	3

**Table.3** Phenol content of *Cassia auriculata*

Sample	Phenol test (mg/g)
Pod (methanol)	148
Leaves (water)	148

**Table.4** Flavonoid test of *Cassia auriculata*

Sample	Flavonoids test (mg/g)
Pod(methanol)	283
Leaves (water)	14

**Table.5** Diameter of Inhibition values (mm) of different extracts of *Cassia auriculata*

Bacterial cultures	Water extract	Methanol extract	DMSO	Disc
<i>E. coli</i>	5mm	5mm	Nil	5mm
<i>P. aeruginosa</i>	8mm	10mm	Nil	10mm
<i>S. aureus</i>	2mm	3mm	Nil	6mm

**Table.6** Diameter of inhibition values (mm) of different extracts of *Cassia auriculata*

Antifungal culture	Water extract	Methanol extract	DMSO
<i>A. niger</i>	1mm	2mm	Nil
<i>T. viride</i>	1mm	2mm	Nil



**Table.7** Determination of bioactive compounds according to the  $R_f$  values calculated through paper chromatography

Sample	Rf Value	Compounds Present
Methanol	0.85	Alkaloids
	0.83	Alkaloids
Water	0.85	Alkaloids
Standard	0.86	Alkaloids

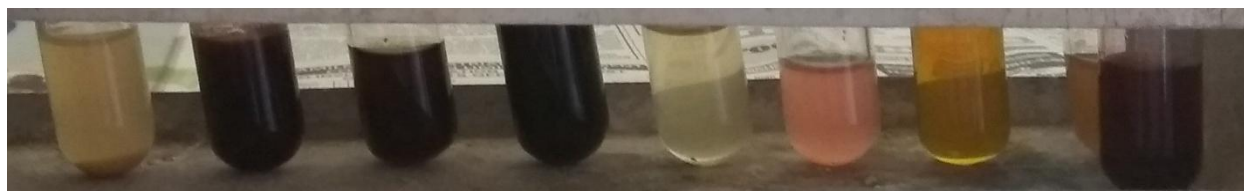
**Table.8** Determination of bioactive compounds according to the  $R_f$  values calculated through Thin Layer Chromatography (TLC)

Sample	Rf Value	Compounds present
Methanol	0.76	Alkaloids
	0.73	Flavonoids
	0.67	Terpenoids
Water	0.76	Alkaloids
Standard	0.71	Ascorbic acid

**Fig.1** Phytochemical analysis of leaves (Methanol)



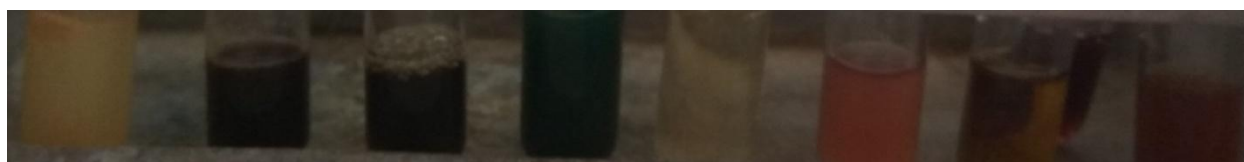
**Fig.2** Phytochemical analysis of leaves (Water)



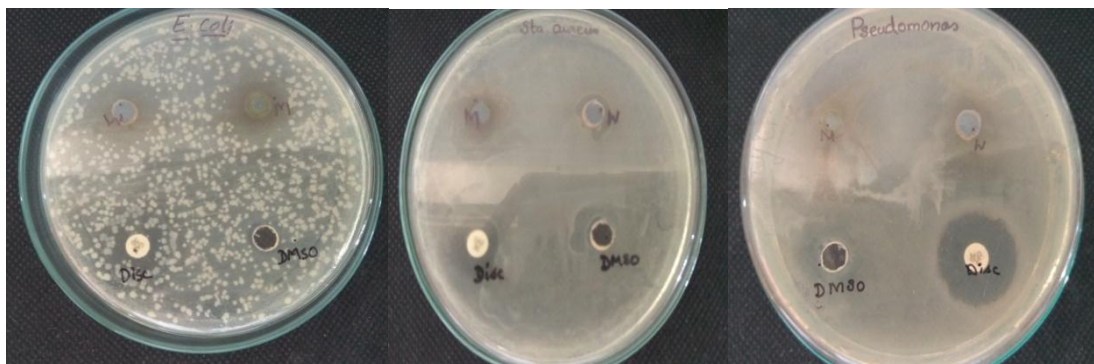
**Fig.3** Phytochemical analysis of pod (Methanol)



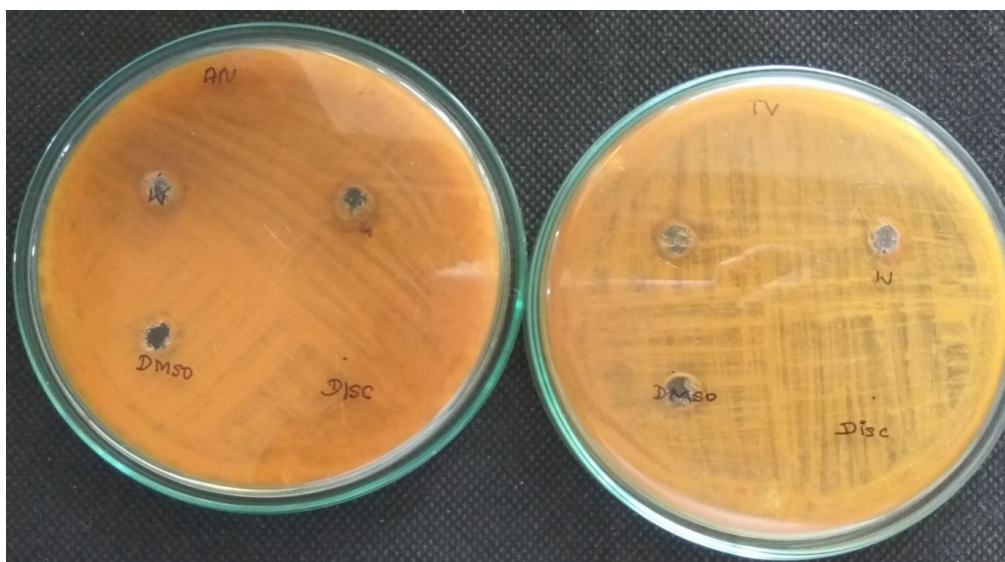
**Fig.4** Phytochemical analysis of pod (Water)



**Fig.5** Antibacterial activity of *Cassia auriculata*



**Fig.6** Antifungal plates of *Aspergillus niger* and *Trichoderma viride*



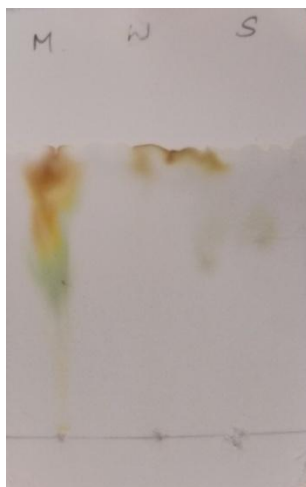
**Fig.7** Result obtained through paper chromatography



M-Methanol Extract, W-Water Extract, S-Standard.



**Fig.8** Result obtained through thin layer chromatography



M-Methanol Extract, W-Water Extract, S-Standard.

### Anti-inflammatory activity

Anti-inflammatory test was done and the plant extract gave significant anti-inflammatory activity as 29.50% for water and 8.36% for methanol at 210nm, with absorbance values. OD value can be obtained as 1.5 for Methanol extract and 1.154 for Water extract (Akah *et al.*, 2003). Here also Methanol extract shows good result than the aqueous extract of leaves

### Chromatography

#### Paper chromatography

Methanol extract of *Cassia auriculata* gives two different type of compounds which is shown in the figure 7.

$R_f$  value for the corresponding solute and solvent can be calculated and the values are shown in the table 7. This technique confirms the presence of Alkaloids.

#### RF values

The retention factor  $R_f$  can be calculated by the following formula,

Distance travelled by the compound  
The Retention Factor  $R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent}}$

### Thin layer chromatography

Methanol extract of *Cassia auriculata* gives three different type of compounds which is shown in the figure 8.  $R_f$  value for the corresponding solute and solvent can be calculated and the values are shown in the table 8. Hence it confirms the presence of flavonoids and terpenoids.

It is quite evident from this is *Cassia auriculata* contains a number of phytoconstituents which reveals its use for various therapeutic purposes. Our results suggests that *Cassia auriculata* is a very good potential source of antioxidant and antimicrobial agents, anti-inflammatory. The methanol extract of pod (so far few research only carried out using pod) has more antioxidant and anti-inflammatory effect than aqueous extract of leaves. Among the two extracts tried pod has produced good result against various pathogens. Paper chromatography and thin layer chromatography confirms the presence of bio

active compounds alkaloids and flavonoids and terpenoids.

### Acknowledgement

We thank Dr. R. Ragunathan, Centre for Bioscience and Nanoscience Research institute Coimbatore, who giving an opportunity to do the dissertation for his valuable advice and guidance for the completion of this work and also thank Dr. R. Ravikumar, Head of the Department, Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam, Tamil Nadu, for providing me an opportunity to do external project.

### References

- Chitme, H.R., Chandra, R., and Kaushik, S., 2003. Studies on anti-diarrheal activity of *Calotropis gigantean* R.Br. in experimental animals. *Journal of Pharmacy & Pharmaceutical Sciences*, 7: 70-75.
- Kim, H.S., 2005. Do not put too much value on conventional medicines. *Journal of Ethnopharmacology*, 100(1-2): 37-39.
- Joy,V.,Paul. John. Peter, M., Yesu. Raj, J., Ramesh, 2012. Medicinal values of Avaram (*Cassia auriculata* linn.): A Review. *International Journal of current pharmaceutical research*, 4(3).
- Ayoola, G.A., Folawewo, A.D., Adesegun, S.A., Abioro, O.O., Adepoju. Bello, A.A., and Coker, H.A.B., 2008. Phytochemical and antioxidant screening of some plants of *Apocynaceae* from south eastern Nigeria. *African Journal of Plant Science*, 2: 124-128.
- Nisha, V., Monisha, C., Ragunathan, R., Jesteena. Johny, 2016. Use of chitosan as edible coating on fruits and in micro biological activity - an ecofriendly approach. *International Journal of Pharmaceutical Science Invention*, 5(8): 07-14.
- Jesteena. Johny, Kannan. Eagappan, Ragunathan, R.R., 2017. Microbial extraction of chitin and chitosan from *Pleurotus* spp, its characterization and antimicrobial activity. *Int J Curr Pharm.* 9(1):88-93.
- Akah, P.A., Nwafor, S.V., Okoli, C.O., 2003. Antiinflammatory activity of plants. *J Nat Remedies.* 3:1-30.
- Emad. Mohamed. Abdallah, 2017. Antibacterial Activity of Fruit Methanol Extract of *Ziziphus Spina-Christi* from Sudan. *Microbiol.App.Sci.*, 6(5): 38-44.
- Homans, A.L., Fuchs, A., 1970. Direct bioautography on thin layer chromatograms as a method for detecting fungi toxic substances. *J Chromatogr.* 51: 327-329.

### How to cite this article:

Monisha, M., M. Sowmiya, R. Ragunathan, Jesteena Johny. 2017. Extraction of Bio Active Compounds from *Cassia Auriculata* Pods and Leaves and its Medicinal Uses. *Int.J.Curr.Microbiol.App.Sci.* 6(8): 425-434. doi: <https://doi.org/10.20546/ijcmas.2017.608.056>