Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(9):479-485

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antibacterial activity of *Cassia auriculata* Linn.

V. Kavimani[1], A. Ramadevi[1], K. Kannan[2], S. Gnanavel[2] and G. Sivaperumal[2]

[1]Department of Chemistry, ACCET, Karaikudi, Tamil Nadu
[2]Department of Chemistry, Government College of Engineering, Salem, Tamil Nadu

---

ABSTRACT

The present study was carried out to evaluate the In Vitro Antibacterial activity of crude extract of locally available plant Cassia auriculata flowers. The current study was performed to screen the phytochemicals that are present in C. Auriculata flowers. To prepare the extract, the shade-dried flowers of C. Auriculata were soaked in water, petroleum ether and methanol. The C. Auriculata flowers extract has several bioactive compounds.The TLC technique has been used to identified the possible compounds present in the methanol extract. The FT-IR spectral data shows functional groups of possible chemical compounds present in the methanol extract of C.Auriculata. The extract was subjected to Disc Diffusion Method to find out the biological activities with three different concentrations [50, 75,100 µl/ml].The methanol extract was only used for this study by using Disc Diffusion Method. Methanol extract exhibited significantly good anti-bacterial activity in a dose-dependent manner.

Keywords: C. Auriculata flowers, phytochemical screening, TLC, FT-IR, Petri Disk Diffusion plate method.

INTRODUCTION

Over the centuries, the use of medicinal herbs has become an important part of daily life despite the progress in modern medical and pharmaceutical research. Approximately 3000 plant species are known to have medicinal properties in India [1]. The Rig-Veda (3700 B.C) mentioned the use of medicinal plants. Our traditional systems of medicines, viz., Ayurveda, Yunani, Siddha and Homeopathy, etc. use herbs for treatment. It is estimated that 40% of the world population depends directly on the plant-based medicine for their health care [2-4]. According to WHO, more than one million people rely on herbal medicines to some extents and also listed 21,000 plants for medicinal uses around the world. India has rich medicinal plant floras of some 25,000 species of these 150 species are commercially used for extracting medicines or drug formulation. In India, the use of medicinal herbs is as old as 1500 BC, underline the medical culture of India both folk traditions as well as codified knowledge system is a deep understanding of the medicinal value of the plants starting with the references in the Ayurveda [5-8]. We have textual evidence of a tradition of use of medicinal plants that is more than 3000 years old. Over the last few years, researchers have aimed at identifying and validating plants derived substances for the treatment of various diseases. Interestingly, it is estimated that more than 25% of modern medicines are directly or indirectly derived from plants. In this context, it worth mentioning that Indian plants are considered a vast source of several pharmacologically active principles and compounds that are commonly used in home remedies for multiple ailments [9-12]. Indian medicinal plants are widely used by all sections of the population, and it has been estimated that several ethnic communities use over 7500 species of plants.

Medicinal plants possess an unlimited and untapped wealth of chemical compounds with high drug potential that make these plants useful as sources of medicines. *Cassia* species have been of keen interest in phytochemical due to their excellent medicinal values. All *Cassia* species are an important rich source of secondary metabolites, notably anthraquinone derivatives and has been used in Chinese and Ayurvedic preparations. Plants have great potential uses, especially as traditional medicine and pharmacopoeia drugs. A large proportion of the world population depends on traditional medicine because of the scarcity and high costs of orthodox medicine [13]. Medicinal plants

have provided the modern medicine with numerous plant-derived therapeutic agents [14]. Many plants contain a variety of phytopharmaceuticals, which have found very important applications in the fields of agriculture, human and veterinary medicine. Natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases [15]. Although in traditional medicine *Cassia* species have been well known for their laxative and purgative properties and the treatment of skin diseases, Still *Cassia* invites the attention of researchers worldwide for its phytochemistry and pharmacological activity. The flowers are used to treat urinary discharges, nocturnal emissions and throat irritation. The flowers are used Ayurveda tradition system widely. Flowers crushed and taken with goat's milk to prevent white discharge in women. The *cassia uriculata* plant contains preliminary phytochemical constituents such as alkaloids, phenols, glycosides, flavonoids, tannins, saponins, proteins, carbohydrates and anthraquinone derivatives handle the pharmacology activity. The plant has been widely used in the traditional system of medicine as a cure for rheumatism [16]. The plant has been reported to possess antipyretic hepatoprotective [17], antidiabetic, anti-peroxidative and antihyperglycaemic [18] and microbicide. C.A*uriculata* commonly known as "avaram". The individual parts of the plant can be used for the treatment of various disorders in humans. Among the different parts, the plant is famous for its attractive yellow flowers that are found to contribute to the various biological activities. The plant has been reported to possess good biological properties like hepatoprotective, anticancer, antioxidant, antidiabetic, anti-inflammatory and antimicrobial properties [19-21]. The present investigation deals with the extraction of essential biological active compounds of *C.Auriculata* linn. This study will help to design the new drugs.

Taxonomic classification

Kingdom	*: Plantae*
Sub Division	*: Spermatophyta*
Division	*: Magnoliophyta*
Class	*: Magnoliopsida*
Sub Class	*: Rosidae*
Order	*: Fabales*
Family	*: Fabaceae*
Genus	*: Cassia*

Traditional Uses: *C. Auriculata.* commonly known as tanner's cassia, also known as "avaram" in Tamil language is a shrub belongs to the Caesal piniaceae family. The shrub is especially famous for its attractive yellow flowers which are used in the treatment of skin disorders and body odour. It is widely used in traditional medicine for rheumatism, conjunctivitis and diabetes. It has many medicinal properties. It is also used for the treatment of ulcers, leprosy and liver disease. The antidiabetic Hypolipidemic, antioxidant and hepatoprotective effect of *Cassia auriculata* have been reported. It was also observed that flower extract of *Cassia* shown to have antipyretic activity.

EXPERIMENTAL SECTION

Collection and Drying of plant materials

The *C. Auriculata* flowers were collected from the Mechari, Salem District, Tamil Nadu as shown in fig.1. The plant materials were air shade-dried and then powdered using an electric blender to get a coarse powder. The powdered samples were kept in sealed containers for extraction purposes.

Fig.1. *Cassia Auriculata* Flowers

Preparation of plant extracts

The air-dried plant material (150 g) was extracted successively with 1500 ml of water and ethanol by using an orbital shaker (soaking method) until a complete extract was effected for 8 hours at room temperature as shown in fig.2.

Fig.2.Orbital shaker for the *C. Auriculata* leaves extraction

The extracts were evaporated to dryness under reduced pressure using a Rotary evaporator and the final crude was stored in a refrigerator at 4°C.

Preliminary Phytochemical screening

The extracts were subjected to preliminary Phytochemical testing to detect the presence of different phytochemical constituents. The plant extract was carried out qualitatively for the presence of Alkaloids, carbohydrates, fixed oils, fats, tannins, gum and mucilage, flavonoids, saponins, terpenoids, lignin and sterols by using the standard method given by (Harborne, 1998) [22].

TLC studies

The aluminium plates precoated with 0.20 mm layers of silica gel $60F_{254}$ (E.Merck, # 1.05570). Additionally, 20x20 cm aluminium plates precoated with 0.20 mm layers of silica gel. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters.

FT-IR Studies

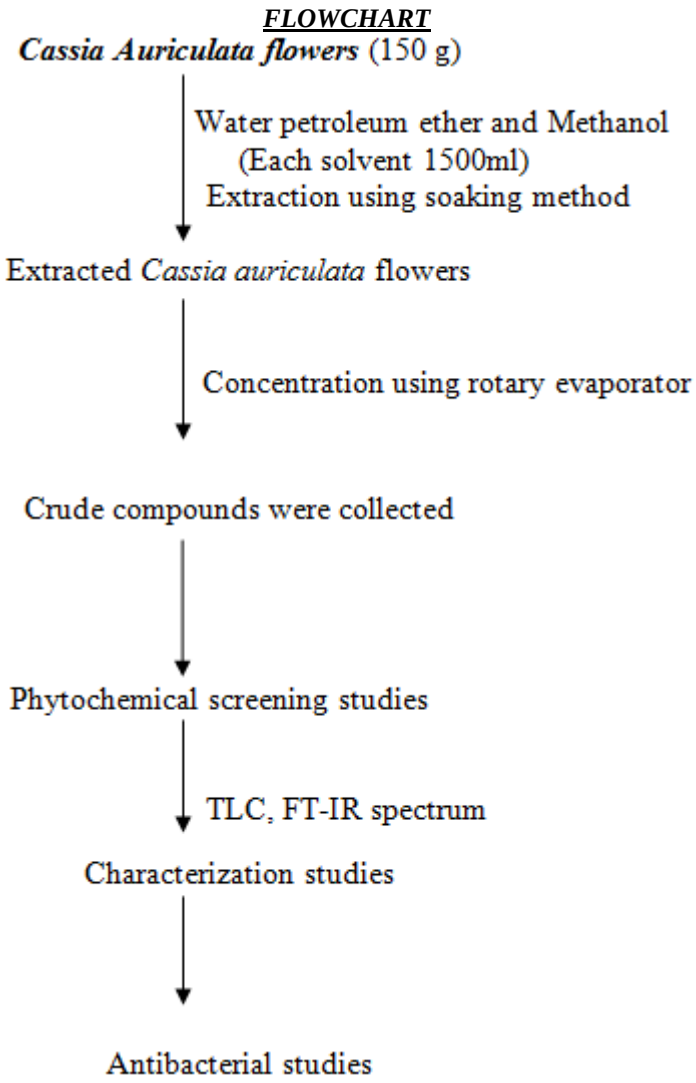
FT-IR spectra were recorded on KBr medium on a Perkin Elmer Rx, spectrophotometer in wave number region 400-4000 cm^{-1}.

Microorganisms Used

The five microorganisms (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas Auregenosa, and Klebsiella pneumonia,*) were used in the entire investigation. The broth cultures of each test organism were prepared by inoculating a loop-full of culture in a 5 ml of nutrient broth and incubated at 37°C for 14 to 16 hours.

Antibacterial assay

Agar well diffusion method was employed as per the modified method of Natarajan et al. [23]. Disc diffusion method. A suspension of test organisms (0.1ml) was swabbed on the Muller Hinton Agar (MHA) by using the sterile cotton swab. After that, a sterile cork borer (5 mm diameter) was used to made wells in the seeded Müller-Hinton agar. Then, 50µl of each extract was separately delivered into wells and allowed to diffuse at room temperature. Equal volumes of DMSO and 25µl of ciprofloxacin (0.1µg/µl) were served as negative and positive control. The plates were incubated at 37°C for 24 hours, and the zone of growth inhibition was measured in mm.



RESULTS AND DISCUSSION

The present investigation has been carried out to evaluate the antibacterial activity of the plant *Cassia Auriculata* for three different solvents extracts. The results of the crude methanol extract obtained using TLC, FT-IR and biological studies are discussed in this present work. The performances of the methanol extract only characterized in the studies.

Phytochemical Screening

The phytochemical analysis revealed that the plants contain bioactive substances that are connected with the antibacterial properties of plants.The presence of alkaloids, Saponin, tannins, flavonoids, and Tannins were determined and included in Table 1.

The test data available in Table .1 indicated that the water extract contains alkaloids, tannins, Coumarin, amino acids and Carbohydrates. The Petroleum ether extract indicates the presence of alkaloids, flavonoids, tannins Coumarin, Carbohydrates and phytosterol. The methanol extract indicates the presence of alkaloids, flavonoids, saponins, Carbohydrates, Terpenoids and phytosterol. It is clearly evident from the table that the other phyto-constituents like phenols, anthraquinone, and glycosides were absent in all the three solvent extracts. These results suggest the presence of primary bioactive metabolite that acts as the precursors for the synthesis of secondary metabolites. These turns help in the development of new bioproducts for future.

TLC analysis

In TLC analysis of the methanol extract of *C. Auriculata* flowers was checked by thin layer chromatography (TLC) on analytical plates over silica gel of 0.2 mm thickness. TLC analysis also suggests the presence of different kinds of phytochemical compounds in flowers extract. Three different spots of compounds have been identified in the TLC

analysis of petroleum ether: ethyl acetate (5:5) and one compound was identified in the TLC analysis of chloroform: methanol (9:1). The reported spots are separated with enough space and having various R_f values showing the presence of possible compounds in the methanol extract a shown in fig.3.

Table-1 Results of Phytochemical screening of different extracts using C. Auriculata flowers

S.No	Test	Water	Petroleum ether	Methanol
1.	**Test for Alkaloids**			
	a) Dragendraffs test	+	+	+
	b) Wagner test	+	+	+
	c) Hagers test	+	+	+
2.	**Test for Flavonoids**			
	a) Lead acetate test	-	+	+
	b) NaOH	-	+	+
3	**Test for Phenols**			
	a) $Fecl_3$	-	-	-
4.	**Test for Tannins**			
	a) $Fecl_3$	+	+	-
	b) $K_2Cr_2O_7$	+	+	-
	c) Lead acetate	+	+	-
5.	**Test for Saponin**			
	a) Foam test	-	-	+
6.	**Test for Amino Acid**			
	a) Xantho proteic test	-	-	-
	b) Biuret Test	-	-	-
7.	Test for Coumarin	+	+	-
8.	Test for Starch (Iodine test)	-	-	-
9.	Test for Quinone	-	-	-
10.	**Test for Carbohydrates**			
	a) Fehling test	+	+	+
	b) Benedict test	+	+	+
	c) Molishs test	+	+	+
11.	**Test for Glycosides**			
	a)Killer –Killani test	-	-	-
12.	**Test for Terpenoids**			
	a) Salkovaki test	-	-	+
	b) Lieberman's test	-	-	+
13.	**Test for Phytosterol**			
	a) Salkovaki test	-	+	+
	b) Lieberman's test	-	+	+
14.	**Test for Anthraquinone**			
	a) Extract +NH_4OH	-	-	-
	b) Benzene Test	-	-	-

+ Present - Absent

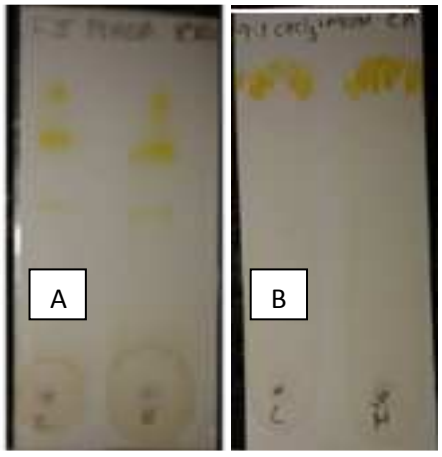


Fig.3. TLC analysis of *C.Auriculata* flower methanol extract
A) Petroleum Ether: Ethyl Acetate and B) Chloroform: Methanol

FT-IR – analysis

FT-IR spectroscopy is most frequently used in phytochemical studies as fingerprint device for comparing natural products. The spectroscopy can also be usefully contributed to structural elucidation when new compounds are

encountered in plants. FT-IR spectra were taken for the methanol extract of *C.Auriculata* flower. The results of the FT-IR spectrum profile are illustrated in the Fig.4. The FT-IR spectrum confirmed the presence of alcohol, phenols, alkenes, alkynes and aromatic compounds in the extract. The spectrum was recorded in the wavelength region between 400 cm^{-1} to 4000 cm^{-1}. The spectrum shows peaks at 3949 $cm^{-1}$and 3772 cm^{-1} alcoholic (strong O-H bonding) which indicates the presence of –O-H stretching of the carboxyl group. These peaks indicate the presence of bonded hydroxyl groups. Further, the peaks observed at 3422 cm^{-1}(O-H stretching) 2239 cm^{-1} represents the stretching bonds of alkenes. The peak observed at 1636 $cm^{-1,}$ and 1564 cm^{-1} represent the C=C aromatic conjugates. The sharp peak at 1415 cm^{-1} and 1115 $cm^{-1}$is assigned to O-H is stretching and C-O stretching (primary alcohol and ester). The peak observed at 659 $cm^{-1,}$ and 486 cm^{-1} represent the presence of different functional groups like Alkanes (-C-H- stretching).

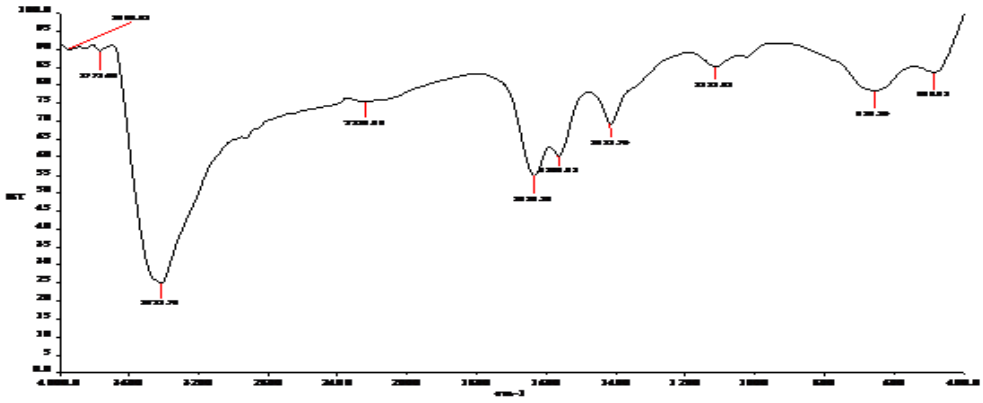


Fig. 4. FT-IR Spectrum of methanol extract of *C.Auriculata* flowers

Antibacterial Activity

The antibacterial activity of the methanol extract of *C.Auriculata* was done by agar well diffusion and disc diffusion methods as shown in fig. 5.

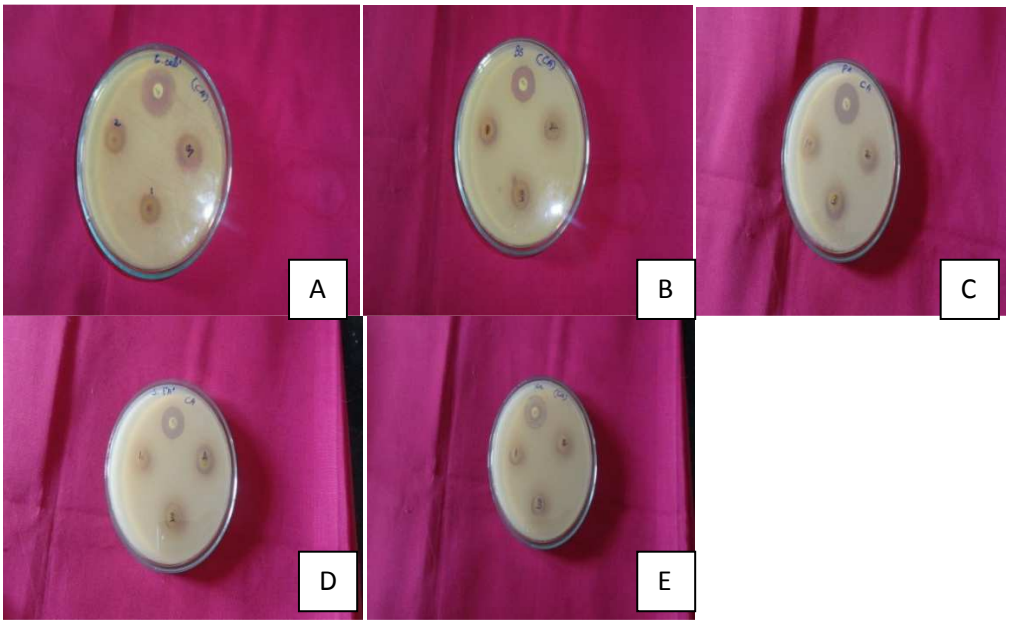


Fig.5. Zone inhibition of *C.Auricullta* flowers using methanol extract against different microorganisms A*) E.Coli, B) Bacillus subtilis* C) *Pseudomonas Auregenosa* D*) Staphylococcus aureus and E) Klebsiella Pneumonia*

The results showed that it is an ideal for the assay of bacterial activities. Antibacterial activity of *C.Auriculata* methanol extract was carried out with clinically isolated pathogens of five microorganisms such as *E.Coli, Bacillus*

subtilis, Pseudomonas Auregenosa, Streptococcus aureus and K. Pneumonia. The results for the antibacterial activity of *C.Auriculata* extract showed a clear zone of inhibition as indicated in Table -2 against on target microbes.

Table -2 Antibacterial activity of methanol extract of *C.Auriculata* flowers

S.No	Microorganisms	Zone of Inhibition (mm) Concentrations (µl/ml)			Control* (µl/ml)
		50	75	100	
1.	*E.Coli*	12	14	17	22
2.	*Bacillus subtilis*	12	13	15	18
3.	*Pseudomonas Auregenosa*	13	14	19	20
4.	*Staphylococcus aureus*	18	19	21	20
5.	*Klebsiella Pneumonia*	11	15	20	20

**Antibiotic*

The concentration (50, 75 and 100 µl/ml) was used. The *C.Auriculata* methanol extract shows antibacterial activity against the entire microorganism was found to be significantly good.

CONCLUSION

It is concluded based on the findings of the present study that the methanol extract of *C. Auriculata flowers* shows good antibacterial activity against bacterial microorganisms such as *E.Coli, Bacillus subtilis, Pseudomonas Auregenosa, Streptococcus aureus* and *K. Pneumonia.* Phytochemical analysis showed that the antibacterial activity of *C. Auriculata flowers* was due to the presence of Phytochemical compounds like alkaloids, flavonoids, tannins, carbohydrates and saponins when compared with other extracts *viz*., petroleum ether, and water . The TLC study of methanol extract indicates the presence of the possible compound. The results of FT-IR analysis from the methanol extract of *C.Auriculata* flowers shows that the peak value at 3772 cm^{-1} alcoholic (strong O-H bonding) which indicates the presence of –O-H stretching of the carboxyl group. The present study justified that the *C. auriculata flowers* in the traditional system of medicine to treat various infectious disease caused by the microbes.

REFERENCES

[1]Tagboto S, *Adv. Parasitol*., **2001**, *50*, 199-295.
[2]Evans WC. Trease and Evans Pharmacognosy, W.B. Saunders Company Ltd., London, **2000**, 19-20.
[3]Newman DJ, Snadder KM. *J. Nat. Prod*., **2003**, *66(7*), 1022 -1037.
[4]A wealth of India. Raw materials, Publications and Information Directorate, New Delhi, Council of Scientific and Industrial Research, **1950.**
[5]Rao, K.N. and Vedavathy, S. *J. Ethnopharmacol*.,**1991**, *33*, 193-196.
[6]Manickam, P., Namasivayam, Asia *Pacific J. Cli. Nut.* **2002**,*11*,157-163,
[7]Pari, L, and Latha, M.. *Cli. Exp. Pharmacol. Physiol*,**2003**, *30*, 38-43**.**
[8] Prakash, S.K. *Int. J. Poultry Sci*.**2006**, *5*, 259-261.
[9]Naidoo K, *African Journal of Pharmacy and Pharmacology*, **2011**, 5(3), 393-397.
[10]oy V, Paul M, Ramesh, *International Journal of Current Pharmaceutical Research*, **2012**, 4, 400-417.
[11] Rajagopal S, Manickam P, *Asia Pacific Journal of Clinical Nutrition*,**2002**, 11(2), 157–163.
[12] Prasanna A, Harish B, *Cell Biology International Journal*,**2009**, 33, 127-134.
[13] Elayarani P, Shanmuganathan S *Asian Journal of Pharmaceutical Sciences*,**2011**, 1(3), 70-72.
[14] Lukmanul H, *International Journal of Diabetes and Metabolism*, **2007**, 15, 100-106.
[15] Gaurav MD, Mukesh DS, *Journal of Pharmaceutical Research & Clinical Practice*,**2011**, 2, 50-58.
[16] Maneemegalai S, Naveen T, *Ethnobotanical Leaflets*,**2010**, 14, 8-20.
[17] Sushma K, Praveen K, *Pakistan Journal of Biological Sciences*, **2012**, 15(9), 408-412.
[18] Kolapo AL, Okunade MB, *World Journal of Agricultural Sciences*,**2009**, 5(1), 90-93.
[19] Horvath EE, Murray CK, *Annals of Surgery*, **2007**, 245(6), 978-85,
[20] Bellik Y, Boukraâ L, *Molecules*, **2013**,18(1), 322-353.
[21] Cathy TH, Checklist for factors affecting wound healing, Advances in Skin & Wound Care, **2011**, 24(4), 192.
[22] Harborne J B. Phytochemical Methods, Chapmann & Hall, London; **1998**, 1-271.
[23] Natarajan D, John Britto S, Srinivasan K, Perumal G. *J Ethnopharmacol*,**2005**, 102(1): 123-126.