Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 7, 2014

Original Article

CHEMICAL COMPOSITION AND CHARACTERIZATION STUDIES OF CASSIA AURICULATA FLOWER EXTRACT

SENTHIL RANI SOUNDHARAJAN[1], RENUKA DEVI PONNUSAMY[1*]

[1]**Department of Biotechnology, Anna University, Regional Centre, Coimbatore 641047, Tamil Nadu, India**
Email: drrenugadevip@gmail.com

Received: 04 June 2014 Revised and Accepted: 14 Jul 2014

ABSTRACT

Objectives: The present study, aimed to determine and characterize the chemical constituents of *Cassia auriculata* flower extract by qualitative, quantitative and analytical techniques.

Methods: Preliminary Phytochemical, total flavonoid and phenol content was determined in the methanolic extract of *C.auriculata* (CAFMEt) using standard methods. C-18 silica gel based column chromatography was used to purify CAFMEt using n-hexane, ethyl acetate and methanol and fraction identified by thin layer chromatography. GC-MS and FT-IR techniques used to characterize the lead fraction.

Results: CAFMEt showed the presence of flavonoid and Phenols in a significant amount. Three fractions was collected from column chromatography *viz*., Fraction 1-3 (n-hexane: yellow) was 2.5mg, (ethyl acetate: light orange) 1.8mg and (methanol: light green) 5.67mg respectively. TLC indicated n-hexane has higher refractive factors 0.457 at yellow band and ethyl acetate fraction has 0.329 at light orange band. 14 chemical constituents were identified by GC-MS included alkanes, alcohol, esters and hydrocarbons. The major peak showed the presence of 4-(4-methylphenoxy) phenol at 22.53%. Infra red spectra revealed the presence of phenolic groups in hexane fraction.

Conclusion: Further studies will be carried out the pharmacological potential of n-hexane fractions of flowers of *C.auriculata.*

Keywords: n-hexane, Ethyl acetate, Flavonoid, FT-IR, Phenol.

INTRODUCTION

The discovery or identification of chemical component from a medicinal plant species forms the basis of therapeutic drug development. In India nearly 7000 medicinal plant species are widely used by the ethnic communities for various alignments [1]. The non-nutritive phytochemical in plants have protective effect against various diseases and disorders [2]. Among the phenolic constituents, the pale yellow and poorly soluble substances such as flavonol, group of flavonoids are widely present in 80% of higher plants [3]. It plays a vital role in attracting the insects and birds for pollination and seed dispersal. Also the toxic water soluble phenolic groups such as simple phenols, hydroxyl benzoic acid and cinnamic acids might serve as allelopathic compounds [4]. Therefore, researchers focused their interest towards herbal medicines in the treatment of diseases because of their minimal side effects and availability [5]. For that, Number of techniques and methods are used to elucidate the dynamic ingredients from the plant origins. Among these, the qualitative and quantitative analysis is very essential for identifying and quantification of active metabolites present in the ethno-medicinally plants which is important for evaluating its therapeutic action and commercial value. Chromatographic and spectroscopic techniques such as Thin layer Chromatography (TLC), Column Chromatography, Gas chromatography coupled with Mass Spectroscopy (GC-MS) and Fourier transform infrared spectroscopy required minimum samples for unambiguous phytocompounds identification [6].

Cassia auriculata L. (Family: Caesalpiniaceae) is an ethno botanically important shrub with attractive yellow flowers and commonly known as "Avaram" in Tamil [7]. The aerial parts of the plant used as a traditional medicine to treat diabetes, conjunctivitis, rheumatism, astringent, antihelminthic, eye troubles, body odor, leprosy and liver disorders diseases [8]. There are some reports available on antidiabetic, acute toxicity, hyperlipidemic, cardioprotective, antioxidant, antimicrobial and hepatoprotective activity [9-10]. Chemical constituents such as protein, carbohydrate, alkaloids, flavonoids and tannin were reported from various parts of the plant [11]. The Kashayam of crushed flowers have been mixed with goats' milk for used to treat white discharge in women and diabetes [12]. There is a lack of scientific data on the quantification and identification of chemical composition in *C.auriculata*. Therefore the present study aimed to determine the phytochemicals, isolate and characterize using chromatographic techniques of CAFMEt.

MATERIALS AND METHODS

Plant Material

Flowers of *Cassia auriculata* was collected from the district of Pudukkottai, Tamil Nadu, India during the month of August-September 2012. The plant material was identified and authenticated by Dr. K.A.A.Kabeer, Scientist C, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India.

Preparation of Flower extract

C.auriculata flowers were shade dried and powdered. 500g of powdered material was extracted with 1500 ml of methanol for 72h. The solvent was evaporated under reduced pressure using rota-evapoator. The final yield of crude extract was used for partial purification.

Qualitative analysis of Phytochemicals

The methanolic flower extract of *C.auriculata* (CAFMEt) used to determine the preliminary phytochemicals was following standard methods [13].

Total Flavonoid Content

Total flavonoid estimation followed by aluminum chloride colorimetric method [14]. Briefly 0.5ml of CAFMEt mixed with 1.5ml (methanol) 0.1ml (10% aluminum chloride), 0.1ml (1M potassium acetate) and 2.8ml of distilled water. The reaction was incubated for 30min at room temperature. The absorbance of the reaction mixture was measured at 415nm. The calibration curve was prepared by using quercetin as a standard.

Total Phenol estimation

The total phenol content of CAFMEt was determined using Folin-ciocalteu (FC) reagent. Briefly, 0.5ml of FC reagent and 5ml of 1M aqueous sodium carbonate was mixed with 0.5ml of CAFMEt. The

reaction mixture incubated for 15min at room temperature and the absorbance was measured at 765nm. The calibration curve was prepared by using catechol as a standard.

Partial Purification using Column chromatography

Column chromatography was performed on a classic 20cm×2cm diameter glass column packed with 15g of silica gel maize size 120cm (HiMedia, Mumbai, India). The methanol solution of the extract (20mL) was applied to the column by use of a pipette and the column was eluted sequentially with n-hexane (fraction1), ethyl acetate (fraction2) and methanol (fraction3).

The column was not allowed to go dry throughout the experiment maintained with respective solvent. Each fraction collected and evaporated to dryness and the residues were dissolved in 5mL respective solvent used for thin layer chromatography.

Thin Layer Chromatography (TLC)

The eluted fractions were used to demonstrate the separation using TLC. Fractions were spotted on the TLC plates. The mobile phase was used as (7:2:1- hexane: ethyl acetate: methanol) with respective proportion as the elution solvent. Retention factor (R_f) values were calculated based on separation different colors of TLC spots.

$$R_f = \frac{\text{Distance travelled by the solvent}}{\text{Distance travelled by the sample}}$$

Gas Chromatography and Mass Spectroscopic analysis

Residue of fractions 1 diluted with appropriate volume of alcohol was performed using a Clarus 500 Perkin Elmer gas chromatography equipped with a Elite-5 capillary column (5% phenyl and 95% dimethyl polysiloxane) (30nm × 0.25mm ID × 0.25µmdf) and mass detector turbomass gold of the company which was operated in EI mode. Helium was the carriers gas at a flow rate of 1ml/min. and the injector was operated at 290ºC and the oven temperature was programmed as follows; 50ºC at 8ºC/min to 200ºC (5min) at 7ºC/min to 290ºC (10min). The identification of unknown phtoconstituents was done by the interpretation of database of National Institute Standard and Technology [15] and Wiley8 library.

Fourier infra red Spectroscopy

A thin disc is prepared under anhydrous conditions from a powder containing about 1mg of fraction1 and 100mg potassium bromide, using a mould and press. The spectral range of measurement is taken from 4000-667cm^{-1} for 3min and recorded [16].

Table 1: Qualitative Phytochemical analysis of CAFMEt

Phytochemicals	Test / Reagent	Methanol Extract
Flavonoids	Shinoda Test	+++
Tannins	Ferric Chloride Test	++
Terpenoids	Salkowski Test	++
Alkaloids	Dragendroffis Test	++
Carbohydrates	Benedicts Test	++
Steroids	Chloroform & Sulphuric acid	++
Coumarins	Fluorescence Test	+
Phenols	Ferric Chloride Test	+++
Saponins	Foam Test	+
Glycosides	Kellerkillani Test	++

Table 2: Chemical composition of n-hexane fraction of *C.auriculata*

Compound Name	Retention Time	Peak Area %	Molecular Formula	Molecular Weight (g/mol)
n-Dodecane	12.567	2.58	$C_{12}H_{26}$	170
Tridecane	12.567	2.58	$C_{13}H_{28}$	184
Pentadecane	12.567	2.58	$C_{15}H_{32}$	212
Tetradecane	12.567	2.58	$C_{14}H_{30}$	198
Heptadecane	16.234	4.62	$C_{17}H_{36}$	240
Eicosane	16.234	4.62	$C_{20}H_{42}$	282
Pentadecane	19.500	2.96	$C_{15}H_{32}$	212
Diethyl phthalate	19.631	1.76	$C_{12}H_{14}O_4$	222
Heptadecane	22.779	1.23	$C_{17}H_{36}$	240
phthalic acid	27.051	6.77	$C_8H_6O_4$	166
4-(4-methylphenoxy)phenol	38.928	22.53	$C_{13}H_{12}O_2$	200
Squalene	40.368	1.44	$C_{30}H_{50}$	410
Hexaene	40.368	1.44	$C_{30}H_{50}$	410
Dibutylchloroethenylsilane	43.940	10.14	$C_{10}H_{20}ClSi$	203
Phenol	48.225	3.48	C_6H_6O	240

Fig. 1: TLC Chamber with isolated fraction of *C.auriculata*

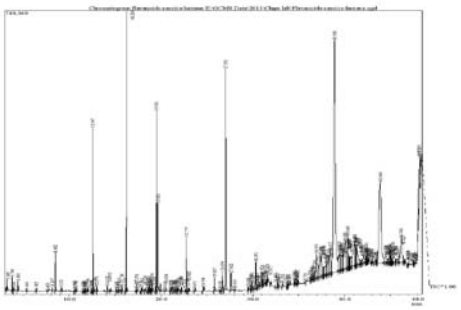

Fig. 2: GC-MS chromatogram of n-hexane fraction of *C.auriculata*

RESULTS

Phytochemical Studies

The CAFMEt showed the significant amount of flavonoids and phenols, followed by tannin, Terpenoids, alkaloids, carbohydrates and steroids (Table 1). The quantitative determination of fraction 1 showed 0.61mg/ml of flavonoid and 0.39mg/ml of phenols respectively.

Chromatographic Studies

There are three fractions was collected from column chromatography. The yield of fraction 1-3 (n-hexane: yellow) was 2.5mg, (ethyl acetate: light orange) 1.8mg and (methanol: light

green) 5.67mg respectively. TLC indicated specific phytoconstituents based on the refractive factor and colour. The n-hexane and ethyl acetate expressed yellow and light orange band due to the presence of xanthophylls (flavonoids). The methanol extract indicated light green band which represented may be steroids. The refractive factor value of n-hexane and ethyl acetate was 0.457 and 0.329 respectively (Figure 1). The refractive factor for n-hexane is higher than ethyl acetate, hence it is used for further analysis.

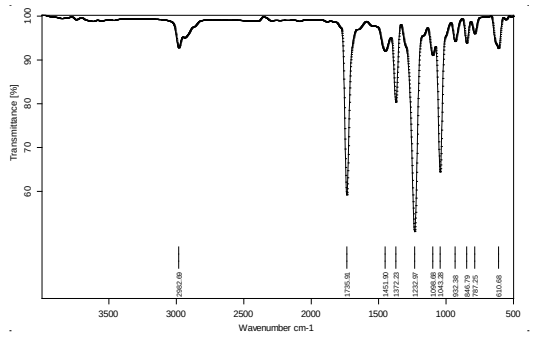

Fig. 3: FT-IR chromatogram of n-hexane fraction of *C.auriculata*

GC-MS chromatogram

GC-MS results revealed the presence of 14 compounds from the Fractions 1 and the peak area ranges from 1.23 to 22.53%. The retention time, percentage of peak area, molecular formula and molecular weight of each compound was showed in Table 2. GC-MS chromatogram was showed in the Figure 2. The identified compounds were broadly divided in to alkanes, alcohol, esters and hydrocarbons. The major peak area showed the presence of 4-(4-methylphenoxy) phenol at 22.53 % referred with NIST library 2011.

FT-IR

FT-IR peaks showed the characteristic peaks of 1735.91cm^{-1} with C=O stretch, 1151.90cm^{-1} assigned C=N stretch, 1372.23cm^{-1} with CH_3 deformations, 1232.97cm^{-1} with P-O-C stretches, 1043.28cm^{-1} with S=O stretches, 932.38 cm^{-1} with CH_2, 846.79cm^{-1} with Sl-H deformation, 784.25cm^{-1} with CH and 610cm^{-1} with S-CN stretches. It showed the presence of phenolic groups in the fraction 1(Figure 3).

DISCUSSION

The present study plant has been tested for its chemical constituents from flowers medicinal plant *C.auriculata*. Earlier reports showed the presence of flavonoids in methanolic leaf extract of *C.auriculata* and absence in petroleum ether and chloroform [17]. In accordance with that, the CAFMEt showed flavonoids in methanol, it acts as a suitable solvent for phytochemical investigation. The presence of xanthophylls in pigment in fraction1, it supported the documents of the presence of yellow color flower in this medicinally valuable species. The decreased order of flavonoid content was observed in leaves extract of *T.vulgaris* in the order of methanol> butanol >chloroform> ethyl acetate>hexane [18]. In contrast with the above statement, Fractions 1 revealed the presence of high amount of flavonoids (0.61 mg/ml) and phenols. It may be depends upon the source of the plant. Every living organism in the world have specific defense system for protection against harmful agents; nearly 80% of the chemical constituents took part in this mechanism of action [19]. Fraction1 derived secondary metabolites might be responsible for earlier reported anti-microbial, antioxidant antidiabetic, activity [20]. Hossain et al [21] pointed the majority of chemical constituents isolated from the hexane extract, which are chemically as well as biologically active. The presence of chemical composition depends also on the nature of parts/ extracts used for identification. The same diethyl phthalate were present at different peak area of 1.76% in *R. mucronata* and 7.32% in fractions 1 of *C. auriculata* [22]. Eicosane, 7-hexyl- obtained at retention time of 60.558 from *Azadirachta indica* by modern sensitive GC-MS [23], in this study, fraction1 showed the Eicosane at retention time of 16.234. Nearly seven major alkanes were designated by GC-MS. Pentadecane could be one of the active compounds responsible for its deterring of ovi position biological activity [24].

CONCLUSION

The secondary metabolites such as flavonoids, phenols, 4-(4-methylphenoxy) phenol, dibutylchloroethenylsilane, pthalic acid and functional groups in n-hexane fractions, which gave the layout for chemical constituents present in the flowers of C.auriculata. Further studies will be conducted to find out the pharmacological action of these constituents.

ACKNOWLEDGEMENTS

The authors wish to thank the authorities of Anna University and BioMed Research Management Services (BRMS), Tamil Nadu, India for providing the necessary support.

CONFLICT OF INTEREST

The authors declared there is no conflict of interest

REFERENCES

1. Sirajuddin SM, Anthropological I. All India Anthropometric Survey:Lakshadweep of India. J Chromatography A 2006.
2. Gurudeeban S. Studies on type 2 diabetes and formulation of tablets from DNA bar-coded Rhizophora apiculata Blume derived Glycosin on Streptozotocin-induced diabetic rats: in vivo, in silico and molecular approaches, Ph.D. Thesis, Annamalai University, Tamil Nadu, India, 2013.
3. Kuhnau J. The flavonoids. A class of semi-essential food components:Their role in human nutrition. In:Bourne GH, editors. World Review Nutrition Diet. Basel: Switzerland; 1976.117–120.
4. Strack D, Dey PM, Harborne JB, London UK. Phenolic Metabolism1997. 387-416.
5. Savithramma N, Rao M, Bhumi G, J. Linga Phytochemical screening of Thespesia populnea (L.) Soland and Tridax procumbens L. J Pharm Res 2011;3:28-34.
6. Chen H, Zuo Y, Deng Y. Separation and determination of flavonoids and other phenolic compounds in cranberry juice by high-performance liquid chromatography. Journal Chromatography A 2001;913(1-2):387-95.
7. Thulasi G, Amsaveni V, Esbl, I. UT, J. Antibacterial Activity of Cassia auriculata against E. J Coli From int Res 2012;3:24-9.
8. Anandan A, Eswaran R, Doss A, Sangeetha G, Anand SP, A. Chemical Compounds Investigation of Cassia auriculata leaves-Folklore Medicinal Plant. Bull Environ Pharmacol Life Sci 2011;1:20-3.
9. Chauhan KN, Patel MB, Valera HR, Patil SD, Surana SJ, J. Hepatoprotective activity of flowers of Cassia auriculata R. Br against paracetamol induced liver injury Rem 2009;9:85-90.
10. Raj J, Peter M, Joy V, J. Yesu Paul John Chemical compounds investigation of Cassia auriculata seeds:A potential folklore medicinal plant. J Asian Sci 2012;2:187-92.
11. Purushotham KN, Annegowda HV, Sathish NK, Ramesh B, Mansor SM. Evaluation of phenolic content and antioxidant potency in various parts of Cassia auriculata L.:a traditionally valued plant. Pakistan J Biol Sci:PJBS 2014;17(1):41-8.
12. Rastogi RP, Mehrotra BN, New C. Compendium of Indian Medicinal plants. Central Drug Research Institute Lucknow and National Institute of Science India 1994.
13. Evans WC. Trease and Evans Pharmacognosy, 14th ed1997.
14. Chang C, Yang M, Wen H, Chem J, J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Drug Anal 2002;10:178-82.
15. Ausloos P, Clifton CL, Lias SG, Mikaya AI, Stein SE, Tchekhovskoi DV, et al. The critical evaluation of a comprehensive mass spectral library. J Am Soc Mass Spectrom 1999;10(4):287-99.
16. Harborne JB. In:Introduction to Ecological Biochemistry, 2nd ed1982.
17. Sachin C, Amit K, J. Phytochemical Analysis and Assessment of In-vitro Anthelmintic Activity of Cassia auriculata Linn leaves. J Am Clin Therapeut 2014;2:161-7.

18. Rota MC, Herrera A, Martinez RM, Sotomayor JA, Jordan MJ. Antimicrobial activity and chemical composition of Thymus vulgaris, Thymus zygis and Thymus hyemalis essential oils. J Food Control 2008;19:681-7.
19. Sukumaran S, Kiruba S, Mahesh M, Nisha SR, Miller PZ, Ben CP, et al. Phytochemical constituents and antibacterial efficacy of the flowers of Peltophorum pterocarpum (DC.) Baker ex Heyne. J Asian Pac J Trop Med 2011;4(9):735-8.
20. Thambidurai M, Rajesh P, Balamurugan B, Kannan VR, J. In vitro antioxidant and anti-microbial of study on Cassia auriculata L. J Int Biol Sci 2010;2:1-7.
21. Hossain MA, Shah MD, Gnanaraj C, Iqbal M. In vitro total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant Tetrastigma from Sabah. Asian Pac J Trop Med 2011;4(9):717-21.
22. Gurudeeban S, Satyavani K, Rajamanickam E, Ramanathan T, Balasubramanian T. Evaluation of Chemical composition and cytotoxicity Effect of Rhizophora mucronata. J Inventi Rapid Med Chem 2013;4:1-5.
23. Hossaina MA, Wafa AS, Afaf A, W. M, J. Toubia Al, Riyamia Al, Identification and characterization of chemical compounds indifferent crude extracts from leaves of Omani neem. J Uni Sci 2013;7:181-8.
24. Victor CG, Jaime PM. Identification of volatiles from *Ceratitis capitata* (Wiedemann) (Diptera:Tephritidae) by means of thermal desorption of solid samples coupled to GC-MS. IOBC WPRS Bull 1999;22:21-25.