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HPTLC analysis of flavonoids among selected members of asteraceae

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Abstract

Flavonoids exhibit a wide range of biological activities and currently are of particularinterest as potential anticancer agents, as insect antifeedants and as natural insecticide. They constitute many secondary metabolites which have polyphenolic properties and with a wide range of pharmacological activities. The present investigation comprises eight medicinal plants which are coming under two sub tribes Heliantheae and Eupatorieae of the family Asteraceae viz., Adenostemma lavenia, O.Kze., Ageratum conyzoides, L., Eupatorium ayapana, Vent., Chromolaena odorata, L., Eclipta prostrata, L., Wedelia chinensis (Osbeck) Merr, Xanthium strumarium, L., and Acanthospermum hispidum, DC. The methanolic extracts of these plants were analyzed using HPTLC CAMAG Linomat 5, Eclipta prostrata, L. is found to have more flavonoid content than others. Rf values of the plants shows the range between 0.63 – 0.68. The data analysis shows that all the plants are positively correlated. The present work emphasizes onto the quantification of flavonoids and emphasizing the therapeutic potential as anti cancerous drug.

Keywords: HPTLC, Flavonoids, Anti cancerous, Polyphenolic, Secondary metabolites.

Introduction

Flavonoids are a vast group of plant secondary metabolites and are commonly seen in flowers, fruits, bark, wine, roots, vegetables, grains, stems and tea[1]. They have variable phenol structures. In the book, "the biochemistry of plants"[2], the importance of flavonoids and other secondary metabolites has been detailed. Quercetin is the most prevalent contributor to the bodily intake of flavonoids. It is mainly found in apples and onions[3-5]. Quercetin has also been reported to prevent renal cell cancer among male smokers[6]. In a case control study conducted between 1994 and 2002 in four Italian areas for studying the relationship between major flavonoid classes and renal cell carcinoma, revealed that flavonols and flavones were inversely related to the risk of renal cancer[7]. The Asteraceae family is well distributed in Indian flora, by its floral structure and chemical composition. It is considered one of the most advanced families from all the dicotyledonous which include about 25,000 species, many of which are rich in secondary metabolites with biological activities[8,9]. A large number of plants belonging to the family Asteraceae contained chemical compounds exhibiting antimicrobial, anticancer and antioxidant properties. About 7,000 compounds had been isolated from some plants of this family. *Ecliptaprostrata*, L. was evaluated for its anticancer activity against Ehrlich Ascites Carcinoma (EAC) in Swiss albino mice. The investigation of the leaves and stems of *Chromolaenaodorata*, L. has showed the presence of essential oils, steroids triterpenes and flavonoids[10-13]. *Xanthium strumarium*, L. extract exhibited anticancer activity against breast MCF7, renal TK10 and melanoma UACC62, human cell lines. The plant was screened by NCI against 60 human cancer cell lines organised into sub-panels representing leukaemia, melanoma, cancer of the lung, colon, kidney, ovary, CNS, breast and prostate[14]. The antifungal and anti-inflamatory effects on methanolic extract of *Wedelia chinensis* (Osbeck) Merr, leaves were studied[15]. The methanolic extract of *Ageratum conyzoides*, L., has antimicrobial activity[16]. The phytochemical study revealed that different flavonoids, coumarinthymoquinone, daphnetin, alpha-terineol are present in the leaf of *Eupatorium ayapana*[17]. Flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation[18] and angiogenesis[19]. The anti-tumor activity of 50% aqueous ethanol extract of *Acanthospermum hispidum* DC against Dalton's ascites lymphoma in mice has also proved[20].

Presently the focus is on the biochemical studies which is carried out to analyze the possible health effects of flavonoids and to assess their activities in the prevention of degenerative diseases along with their therapeutic value as potential drugs. Objectives of the present study are to emphasize onto the quantification of flavonoids and emphasizing the therapeutic potential as anti cancerous drug.

Materials and methods

The plants used in the present study were collected and authenticated. The materials were washed and air dried under shade for two weeks. The dried plant parts, finely powered using electric grinder, sieved and subjected for the extraction. Powered samples were extracted with 200 ml of methanol for 8-12 hrs by the cold extraction method. Quantitative phytochemical characterization was done using High Performance Thin Layer Chromatography[21,22].

For this, CAMAG HPTLC system equipped with Linomat 5 applicator, TLC scanner 3 and WIN CATS software were used. High Performance Thin Layer Chromatography were performed on silica gel 60F254 (10cmx10cm; Merck). Methanol extract of the selected plants (10mg/ml) and collected fractions residue 1mg/ml) was subjected to HPTLC (CAMAG, Switzerland) analysis. Extract and each fraction were spotted on a silica gel 60F254 TLC plate. The plate was air dried and then developed by using the solvent system Toluene: ethyl acetate: formic acid: methanol (5.5:4:1:0.5) as mobile phase in a CAMAG twin – trough glass chamber (20x10cm) previously saturated with mobile phase vapor for 20 min. After developing the plate, it was dried at 105 ^{0}C for 5 min and then it was scanned using Scanner 3 (CAMAG, Switzerland) at 366 nm using Win CATS software. Chromatograms were evaluated before and after spraying with Anisaldehyde sulphuric acid reagent.

Estimation of total flavonoids: Total flavonoids content was measured by aluminium chloride chlorimetric assay. An aliquot (1ml) of extract or standard solution of catechin (20, 40, 60, 80, 100 mg/l) was added to 10ml volumetric flask containing 4ml of distilled water to the flask was added 0.3 ml 5% $NaNO_2$ (Sodium nitrate).After 5 m inutes 0.3 of 10% Al_2Cl_3 (Aluminium chloride) was added. At the 6th minute, 2ml1M NaOH was added (40gms in 1000ml) and the total volume was made upto 10ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. The total flavanoid content was expressed as mg catechin equivalence (CE/100 gm) fresh mass.

Results and discussion

Phytochemical screening on methanolic extracts of the selected plants revealed the presence of flavonoids. The increasing effects of flavonoids have become known over the years through the discovery of new plant flavonoid and their derivatives. Various solvent compositions of the mobile phase for HPTLC analysis were examined in order to achieve high resolution and reproducible peaks. The mobile phase with the composition of Toluene: ethyl acetate: formic acid: methanol (5.5:4:1:0.5) showed high resolution and repeated results confirmed their efficiency and accuracy. Quercetin was used as a standard and the area percentage of quercetin was found to be 94.84% by HPTLC (Figure-1). The methanolic extracts of the two plants studied shows the presence of quercetin, ranges from 0.63 – 0.68. Spots with Rf values -0.01, 0.14, 0.18, 1.04 is common in the studied plants prove that the presence of similar compounds and their chemical similarity as a single family. *Eclipta prostrata*, L. gives the maximum area of 8541.4 and that of height 323.7. These plants shows positive correlation with each other (Figure-2). The highest correlation is shown between *Xanthium strumarium*, L. and *Acanthospermum hispidum*, DC. and lowest is between *Wedelia chinensis* (Osbeck) Merr and *Adenostemma lavenia*, O.Kze. (Table-3). Therefore these plants have high medicinal importance but their potentials are not fully explored. The anti cancerous properties of the above plants were may be due to the presence of flavonoids. This has to be further proved through cell line cultures and animal study. The present study proves that the selected plants can be used as an anticancerous drug along with other formulations of drugs.

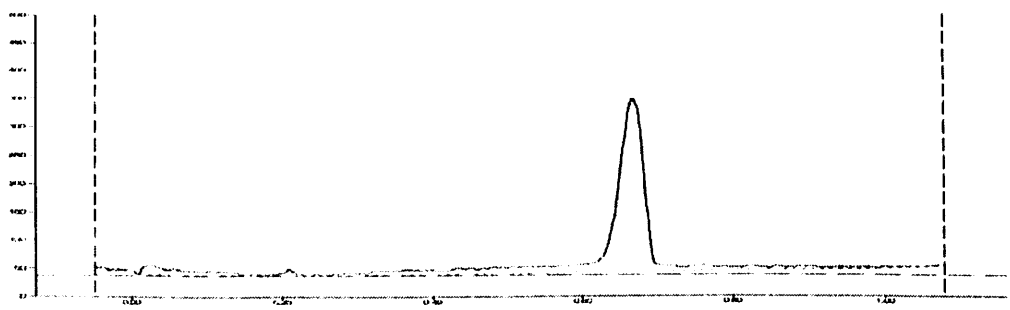

Figure-1: HPTLC Chromatogram of Quercetin.

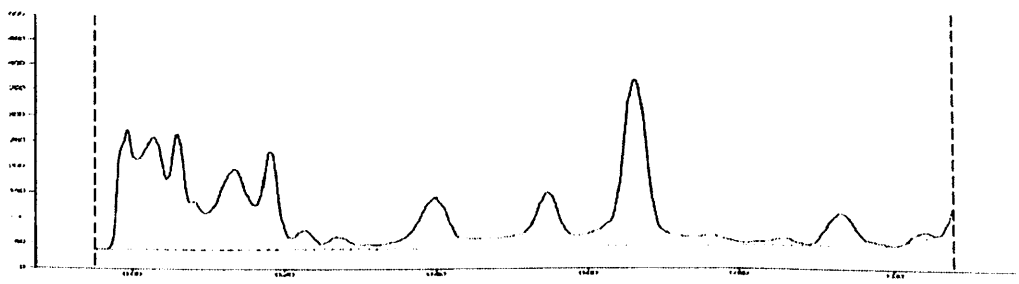

Figure-2: HPTLC Chromatogram of *Ecliptaprostrata*, L.

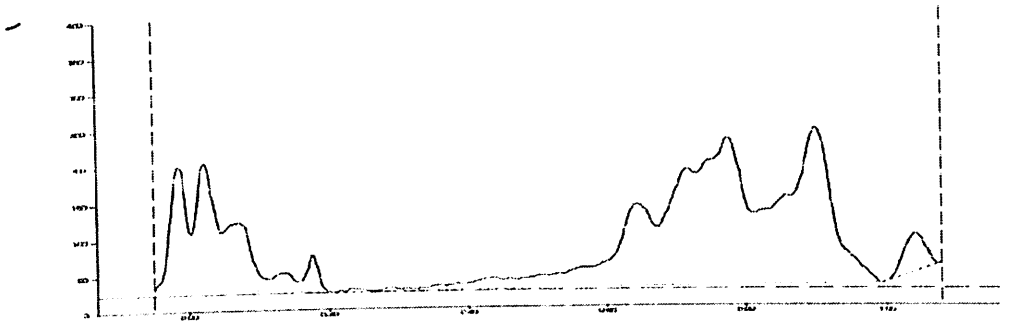

Figure-3: HPTLC Chromatogram of *Chromolaena odorata*, L.

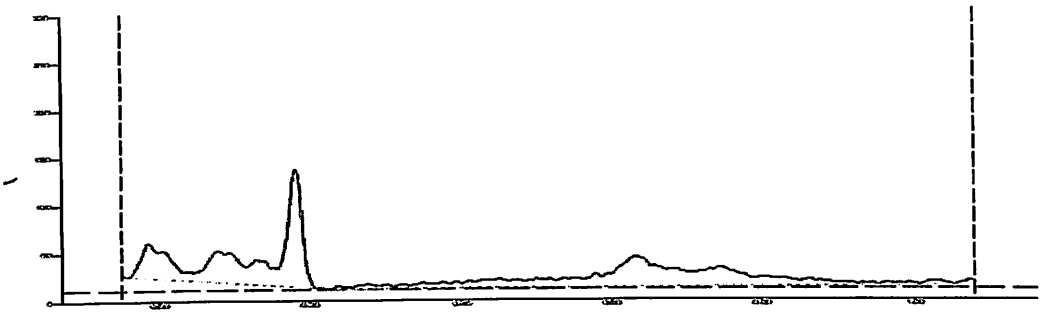

Figure-4: HPTLC Chromatogram of *Xanthium strumarium*, L.

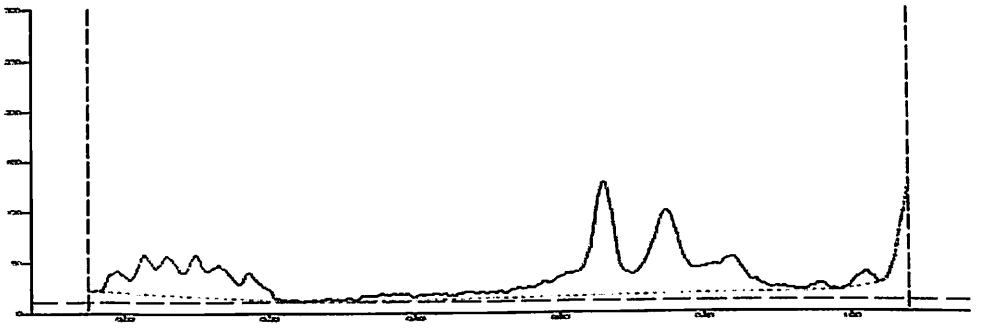

Figure-5: HPTLC Chromatogram of *Acanthospermum hispidum*, DC.

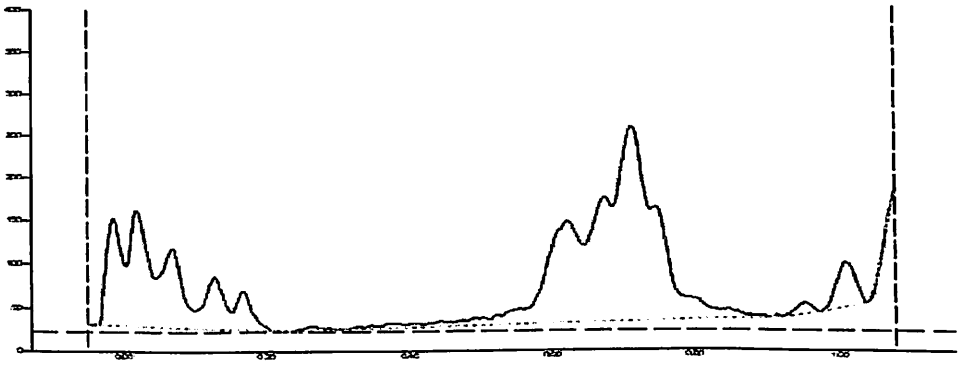

Figure-6: HPTLC Chromatogram of *Ageratum conyzoides*, L.

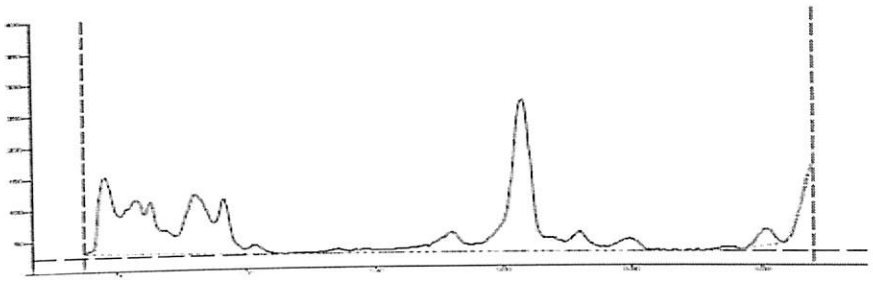

Figure-7: HPTLC Chromatogram of *Wedelia chinensis* (Osbeck)Merr.

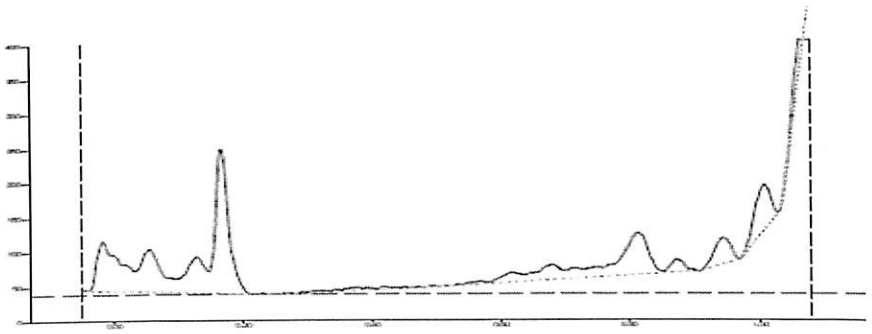

Figure-8: HPTLC Chromatogram of *Adenostemma lavenia*,O.Kze.

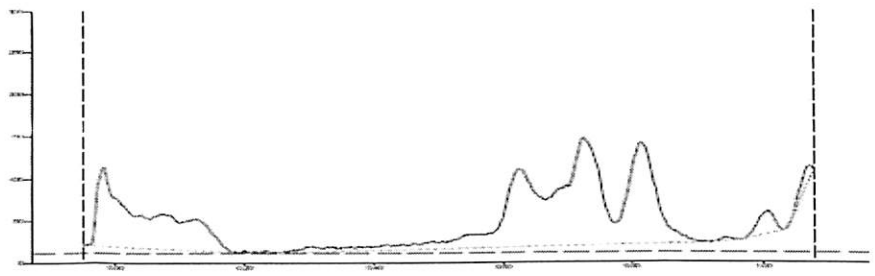

Figure-9: HPTLC Chromatogram of Eupatorium *ayapana*,Vent.

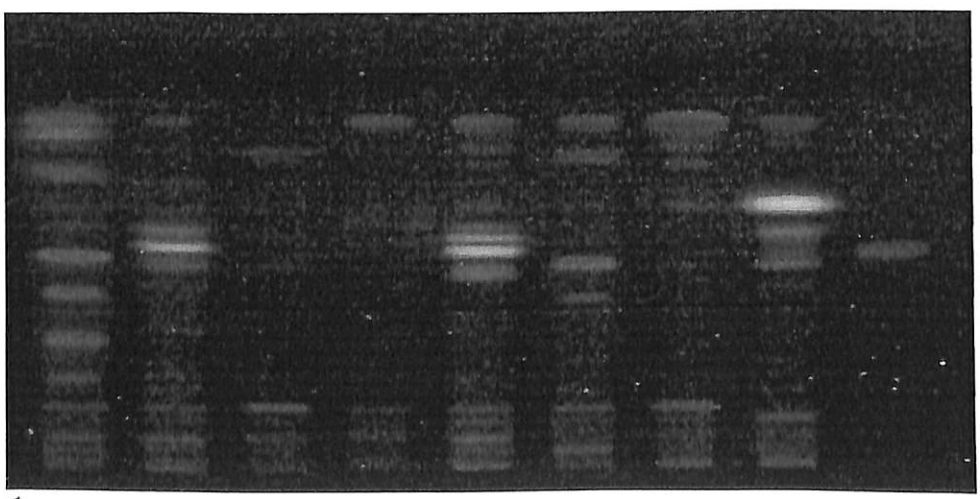

Figure-10: Developed HPTLC plate prior to derivatization under UV 366 nm.

Table-1: HPTLC –Comparative chart of Peak, Max. height and Area of Flavonoid profile of themethanolic extracts.

No.	Sample	Max. ht	Max. Rf	Area	Area %
1	Quercetin	301.1	0.67	7143.9	94.84
2	*Eclipta prostrata*, L.	323.7	0.66	8541.4	22.05
3	*Chromolaena odorata*, L.	110.8	0.65	4333.8	11.74
4	*Xanthium strumarium*, L.	29.63	0.64	1052.9	20.46
5	*Acanthospermum hispidum*, DC.	110.5	0.66	3097.5	28.79
6	*Ageratum conyzoides*, L.	142.8	0.68	3068.8	12.42
7	*Wedelia chinensis* (Osbeck)Merr.	236.1	0.63	5519.7	32.26
8	*Adenostemma lavenia*, O. Kze.	21.3	0.68	459.0	4.04
9	Eupatorium *ayapana*, Vent.	92.6	0.63	3220.5	20.01

Table-2: Total Flavanoid content among the plant samples.

Sl.no	Sample	Total flavanoid mg/gm
1.	*Eclipta prostrata*,L.	27.95 ± 0.09
2.	*Chromolaena odorata*, L.	31.72 ± 0.15
3.	*Xanthium strumarium*, L.	23.4 ± 0.25
4.	*Acanthospermum hispidum*,DC.	23.63 ± 0.40
5.	*Ageratum conyzoides*,L.	22.8 ± 0.15
6.	*Wedelia chinensis* (Osbeck)Merr.	22.07 ± 0.21
7.	*Adenostemma lavenia*,O.Kze.	19.82 ± 0.46
8.	*Eupatorium ayapana*,Vent.	17.06 ± 0.01

Table-3: Correlation of the total flavonoids among the plant samples.

	EP	*CO*	*XS*	*AH*	*AC*	*WC*	*AL*	*EA*
EP	1							
CO	0.940055	1						
XS	0.997547	0.91388	1					
AH	0.997875	0.915836	0.999988	1				
AC	0.985364	0.868166	0.994879	0.994378	1			
WC	0.817757	0.965016	0.775464	0.778514	0.707677	1		
AL	0.97923	0.851387	0.99102	0.99036	0.99946	0.684074	1	
EA	0.877584	0.988471	0.841874	0.844479	0.783017	0.993589	0.762152	1

EP-*Eclipta prostrata*, L., CO-*Chromolaena odorata*, L., XS- *Xanthium strumarium*, L., AC-*Acanthospermum hispidum*, DC., AC- *Ageratum conyzoides*, L.,WC-*Wedelia chinensis* (Osbeck) Merr, AL-*Adenostemma lavenia*, O.Kze., EA- *Eupatorium ayapana*, Vent.

Conclusion

The present research work comprises of the estimation of total flavonoids using UV Spectrophotometry and quantitative phytochemical characterization using High Performance Thin Layer Chromatography. From the above results, it is evident that the studied plants are rich in flavonoids. Flavonoids are generally nontoxic and manifest a diverse range of beneficial biological activities. The role of flavonoids in cancer prevention is widely discussed. There is much evidence that flavonoids have important effects on inhibiting carcinogenesis. So we can infer that the studied plants have reasonable medicinal properties that have to be revealed through further studies. By finding out the therapeutic potency of these medicinal plants, it will provide a rationale for their use as anti-cancerous medicines as they are rich in flavonoids. It would be a good basis for further pharmacological studies and conservation of wild varieties of plants for the future benefit of mankind.

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References

1. Nijeveldt R., Nood E.V., Hoorn D.E.C.V., Boelens P.G., Norren K.V. and Leeuwen P.A.M.V. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. *Am J ClinNutr*, 74(4), 418-425.
2. Stumf P.K. and Corm E.E. (1981). Biochemistry of Plants. Academic Press. London.
3. Knekt P., Jarvinen R., Reunanen A. and Maatela J. (1996). Flavonoid intake and coronary mortality in Finland: a cohort study. *Br Med J*, 312(7029), 478-481.
4. Gibellini L., Pinti M., Nasi M., Montagna J.P., De Biasi S., Roat E., Bertoncelli L., Cooper E.L. and Cossarizza A. (2011). Quercetin and cancer chemoprevention. *Evid Based Complement Alternat Med*, 591356.
5. Giuliani C., Noguchi Y., Harii N., Napolitano G., Tatone D., Bucci I., Piantelli M., Monaco F. and Kohn L.D. (2008). The flavonoid quercetin regulates growth and gene expression in rat FRTL-5 thyroid cells. *Endocrinology*, 149(1), 84-92.
6. Wilson R.T., Wang J., Chinchilli V., Richie J.P., Virtamo J., Moore L.E. and Albanes D. (2009). Fish, vitamin D and flavonoids in relation to renal cell cancer among smokers. *Am J Epidemiol*, 170(6), 717-729.
7. Bosetti C., Rossi M., McLaughlin J.K., Negri E., Talamini R., Lagiou P., Montella M., Ramazzotti V., Franceshi S. and LaVecchia C. (2007). Flavonoids and the risk of renal cell carcinoma. *CancerEpidemiol Biomarkers Prev*, 16(1), 98-101.
8. Gupta M.P. (1995). *Plant* as medicinal esiberoamericanas. Bogota.*CYTED-SECAB*, 270, 324.
9. Okunade A.L. (2002). Review Ageratum co-nyzoides L. (Asteraceae). *Fitoterapia*, 73, 1-16.
10. Inya-Agha S.I., Oguntimein B.O., Sofowora A. and Benjamin T.V. (1987). Phytochemical and Antibacterial Studies on the Essential Oil of Eupatorium odoratum. *Int. J. Crude Drug Res*, 25(1), 49-52.
11. Suksamram A., Chotipong A., Tananit S., Boongird S., Timsuksai P., Vimuttipong S. and Chuaynugul A. (2004). Antimycobacterial Activity and Cytotoxicity of Flavonoids from the Flowers of Chromolaena odorata. *Arch Pharm Pres.*, 27(5), 507-511.
12. Talapatra S.K., Bhar D.S. and Talapatra B. (1974). Flavonoid and TerpenoidConstitiuents of Eupatorium odoratum. *Phytochemistry*, 13(1), 284-285.
13. Bose P.K., Chakrabarti P., Chakrabarti S., Dutta S.P. and Barua A.K. (1974). Chemical Examination of the Leaves of Eupatorium odoratum. *Trdans. Bose Res. Ins. (Calcutta)*, 37, 25-30.
14. Fouche G., Cragg G.M., Pillay P., Kolesnikova N., Maharaj V.J. and Senabe J. (2008). In vitro anticancer screening of South African plants. *J Ethnopharmacol*, 119(3), 455-461.
15. Manjamalai A., Shukoor A.S., Haridas A. and Berlin G. (2011). Evalution of antifungal and anti- inflammatory effects on methanolic extract of Wedeliachinensis. *Int. J. Pharm. Biol. Res*, 3, 30-37.
16. Almagboul A.Z., Farrog A.A. and Tyagi B.R. (1985). *Fitoterapia*, 56, 103.
17. Kawase Masami, Sakagami Hiroshi, Motohashi Noboru, Hauer Hermann, Chatterjee Shyam S., Spengler Gabriella, Vigyikanne Aniko Varadi, Molnar Annamaria and Molnar Joseph (2005). Coumarin derivatives with tumor-specific cytotoxicity and multidrug resistance reversal activity. *In Vivo*, 19(4), 705-711.
18. Weber G., Shen F., Prajda N., Yeh Y.A., Yang H., Look K.Y. and Herenyiova M. (1996). Increased signal transduction activity and down-regulation in human cancer cells. *Anticancer.Res*,16(6A),3271-3282.
19. Fotsis T., Pepper M.S., Aktas E., Breit S. and Rasku S., Adlercreutz H. (1997). *Cancer.Res*, 57, 2916-2921.
20. Deepa N. and Rajendran N.N. (2007). Anti-tumor Activity of Acanthospermumhispidum DC on Dalton Ascites Lymphoma in Mice. *Natural Product Sciences*, 13(3), 234-240.
21. Ramya V., Dheena D.V. and Umamaheswari S. (2010). In vitro studies on antibacterial activity and separation of

active compounds of selected flower extracts by HPTLC. *J Chem Phar Res*, 2(6), 86-91.

22. Sethi P.D. (1996). High Performance Thin Layer Chromatography: Quantitative Analysis of Pharmaceutical Formulations. CBS Publishers and Distributers: *New Delhi*, 10-60.