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Research Article

DEVELOPMENT OF TLC AUTOGRAPHICALLY GUIDED GC-MS PROFILE OF MAJOR PHYTOCHEMICAL CONSTITUENTS FOR COMPARATIVE ASSESSMENT IN DIFFERENT EXTRACTS OF *AGERATUM CONYZOIDES* L.

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ABSTRACT

Objective: In present study, a comparative study of TLC profile of three extracts (Dichloromethane, Methanol and *n*-Hexane) has been carried out for visible screening of phyto-chemical constituents. In these extracts (medium polar, polar and non polar) of *A. conyzoides* L.(aerial part), eleven phyto-chemical constituents are assessed by GC-MS in different concentrations.

Methods: 1g of powdered *A. conizoides* L. (aerial part), were soaked overnight separately in 10 ml each of DCM, methanol and *n* - hexane. The plant extracts were continuously stirred for 6 hrs and kept up to 18 hrs at room temperature and filtered. The solution so obtained is subjected to TLC and GC-MS study.

Results: In TLC study a number of different bands appeared at 254nm & 366nm. After derivatization, in white light there are nine bands are present in these three extracts. There are some bands which have similar R_f values in these three extracts. The overall highest peak area present in these three extracts (63.05%) is precocene-II (RT-12.882) and the lowest peak area (0.38%) present in hexadecanoic acid (RT-17.127) in DCM extract.

Conclusion: The comparative study of GC-MS shown that the concentration of phyto - constituents germacrene-D, caryophyllinoxide, precocene-I, citrolinol and *trans* - squalene in these three extracts are found in the order of *n*- hexane > DCM > methanol. It has been concluded from this study that quantitative comparative assessment of important phyto-constituents is useful for researchers to select a particular enriched extract for therapeutic efficacy.

Keywords: *Ageratum conyzoides* L., Phyto-chemical constituents, *trans*-Squalene, β-Farnesene, TLC, GC-MS.

INTRODUCTION

Ageratum conyzoides L. is most common and widely spread over the world, especially in the tropical and subtropical regions. It is mainly found in West Africa and some parts of Asia as well as South America. The medicinal plant is annual branching herb which grows up to 1 m in height.

The stems and leaves are covered with fine white hairs while the leaves are ovate and up to 7.5 cm long. The flowers are in different colour from purple to white, less than 6 mm across and arranged with terminal inflorescence. The fruits are achene and easily dispersed because of its propagation it becomes a weed and causes problems for farmers and ecologists [1]. It is not eaten by men because of its bad odour, like a male goat and is named goat weed or billy goat weed.

The whole plant is only used for medicinal purposes and has a long history in the folk medicine of different countries. *A. conyzoides*, a widespread weed in Colombia and in other countries, has been used in folk medicine to cure several diseases and also as an insecticide. These products promote physiological changes in the insects that include precocious metamorphosis, sterilization, inhibition of sex pheromone production, embryo genetic damage, and interrupted circadian feeding rhythms or diapauses induction [1- 3]

MATERIAL AND METHODS

Collection of plant material

The aerial part of the plant *A. conyzoides* L. was collected around Itawa, Utter Pradesh. The medicinal plant is identified and authenticated by Biosciences Division of Institute of Pesticide Formulation Technology (IPFT), Gurgaon, Haryana.

Sample preparation

The aerial part of the plant was dried in shade to avoid loss of essential oils. Dried plant part was powdered in a mixer grinder. Powdered drug was extracted from solvents range from polar to non polar i.e. dichloromethane, methanol and *n*-hexane in the ratio of 1:5 (one part drug: five part solvent) by hot extraction method for six hours. The temperature of soxhlet extractor is 40 ^{0}C. All crude extracts were filtered and concentrated in vacuum at 40 ^{0}C using rotatory evaporator. The concentrated extract was then dried aseptically with the help of drier.

Thin Layered Chromatography Methodology

1g of powdered *A. conizoides* L. (aerial part), were soaked overnight separately in 10 ml each of DCM, methanol and *n* - hexane. The plant

extracts were continuously stirred for 6 hrs and kept up to 18 hrs at room temperature and filtered. 10 µl of the these three extracts were applied on Merck aluminum pre-coated plate with silica gel $60F_{254}$ of 0.2 mm thickness through Linomat V. The plate was developed in solvent system of *toluene: ethylacetate (9:1)*. The plate was dried and visualized in UV 254 & 366 nm. The plate was dried and derivatized by anisaldehyde - sulphuric acid reagent and heated in 105 ^{0}C. Again the plate was visualized in white light and there a number of bands are shown in the plate.

Gas Chromatography–Mass Spectroscopy Methodology

For GC-MS analysis of the extracts GC-MSD Shimadzu (Mass Selective Detector, GC-QP 2010 plus MSD model) equipped with DB-5MS fused silica capillary column (Agilent J & W GC column, 5% Phenylated methyl siloxane, 30 m length × 0.25 mm i.d. × 0.25 µm film thickness) was used. Analysis was carried out using oven programming of initial temperature 50 ^{0}C for 2 min followed by a ramp rate of 20 ^{0}C /min up to a temperature of 130 ^{0}C followed by 12 ^{0}C /min ramp to a temperature of 180 ^{0}C with a hold time of 10 min. The injector temperature was set at 280 ^{0}C in split less mode; solvent delay time was given as 6.5 min. The interface and ion source temperatures were set at 280 ^{0}C and 250 ^{0}C, respectively. MS Quadruple temperature was set at 150 ^{0}C, an emission current of 300 µA, lower vaccume 3.0e + 000 Pa and upper vaccume < 1.0e - 004 Pa. The instrument was operated in Electron Impact Mode (EI) with electron energy 70 eV, carrier gas helium was used at a flow rate of 1 mL/min. For confirmation of analytes analysis was done by SIM mode. The compounds were identified by comparing the mass

spectral data with that of in-house library already stored in a compact library of chemical substances (NIST library).

RESULT AND DISCUSSION

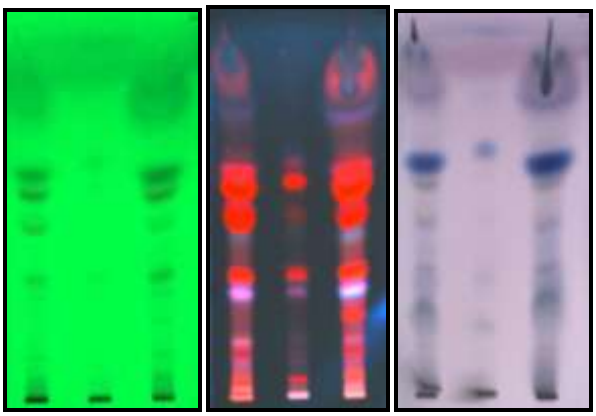
Phyto-chemical screening

The preliminary phytochemical screening of plant aerial part in three extract was carried out as per methods and tests by Harborne J. B. [4]. The analysis showed that the presence of bioactive compounds like flavonoides, alkaloids, cumarines, essential oils, chromenes, benzofurans, terpenoides and tannins. The main chemical constituents present in aerial part is 7-dimethoxy-2,2-dimethylchromene, 6-demetoxyageratotochromene, 6-vinyl-demethoxyageratotochromene, ageratotochromene, α - cubebene, α - pinene, α - terpinene, β -caryophyllene, β - cubene, β - elemene, β - farnesene, β - myrcene, β - pinene, selinene, sitosterol, cardinene, caryophyllene-oxide, conyzorigin, coumarin, dotriacontene,

endoborneol, endo-bornyl-acetate, ethyl-eugenol, ethyl-vanillin, farnesol, friedelin, hydrogen-cyanide, hexadecenoic-acid, Kaempferol, Kaempferol-3,7-diglucoside, Kaempferol-3-O-rhamnosylglucoside, linoleic-acid, quercetin, quercetin-3,7-diglucoside and quercetin-3-O-rhamnosylglucoside [5, 6, 7-10].

Comparative study of TLC chromatogram

On comparative study of TLC chromatogram, it has been observed that a number of different bands appeared at 254nm & 366nm. After derivatization, in white light there are nine bands are present in these three extracts, shown in Figure 1. There are some bands which have similar R_f values in these three extracts as shown in Table 1**.** This may leads to identify and estimate these different chemical constituents by GC-MS.



1 2 3 1 2 3 1 2 3

UV 254 nm UV 366nm after derivatisation (White light)

Fig. 1: TLC chromatogram of DCM, Methanol, *n*- Hexane extracts of aerial part of *Ageratum conizoides* L.

Solvent system: *Toluene: Ethyl acetate (9:1)*

Track 1: DCM extract - 10 µl, **Track 2**: Methanol extract - 10 µl, **Track 3**: n-Hexane extract - 10 µl

Table 1: R_f Values of three different extracts of aerial part.

Samples	Visualization / Detection (R_f Values)		
	UV 254 nm	UV 366 nm	White Light
Track 1	0.32, 0.45, 0.54, 0.60	0.07, 0.12, 0.29, 0.33, 0.48, 0.57	0.25, 0.47, 0.56, 0.61
Track 2	0.34, 0.62	0.07, 0.29, 0.33, 0.48, 0.57	0.20, 0.65
Track 3	0.22, 0.34, 0.47, 0.54, 0.60	0.07, 0.12, 0.22, 0.33, 0.48, 0.57, 0.71	0.28, 0.35, 0.63

GC-MS analysis

GC – MS analysis was carried out on three extract (DCM, methanol and *n*-Hexane) of *A. conizoides* L. After analysis, it is found that eleven major phyto-constituents present in the aerial part of medicinal plant. In this analysis, we have detected a number of phyto-constituents present in the plant part *i.e.,* (+) β - fenebrene, germacrene - D, *trans* – (β) caryophyllene, β - farnacene, caryophyllinoxide, precocene-I, precocene-II, hexadecanoic acid methyl ester, α - linolinic acid, citrolinol and *trans* - squalene. Out of eleven phyto-constituents, five phyto-constituents namely germacrene - D, caryophyllinoxide, precocene - I, citrolinol and *trans* - squalene are found having highest concentration in *n* - hexane extract as compared to DCM and methanol extract. The compounds (+) β - fenebrene, hexadecanoic acid methyl ester and α - linolinic acid are in higher concentration present in alcoholic extract while *trans* – (β) caryophyllene, β - farnacene and precocene-II are present in more concentration in DCM than the methanolic and *n* - hexane extract. In dichloromethane extract, the highest peak area (63.05 %) is present in precocene-II (RT- 12.882) while the lowest peak area (0.38 %) present in hexadecanoic acid (RT- 17.127). In alcoholic extract, the highest peak area (57.08 %) are present in precocene-II (RT- 12.853), however, the lowest peak area (0.49 %) present in citrolinol (RT-21.127). In *n* - hexane extract the highest peak area (51.70 %) are present in precocene-II (RT- 12.886) and the lowest peak area (0.51 %) present in (+) (β)-fenebrene (RT- 9.470).

The overall highest peak area present in these three extracts (63.05 %) is precocene-II (RT-12.882) and the lowest peak area (0.38 %) present in hexadecanoic acid (RT-17.127) in DCM extract. The detail results of GC-MS analysis has been given in **Table 2** and the total ion chromatography (TIC) showing the peak identity of the compounds identified have been shown in **Figure 2, 3 and 4.**

Table 2: Details of Phyto-constituents identified form three extracts of aerial part of *A. conizoides* L. by GC-MS

S. No.	Phyto-constiuents	DCM extract		Methanol Extract		Hexane extract	
		Retention time	Peak area (%)	Retention time (RT)	Peak area (%)	Retention time (RT)	Peak area (%)
1.	(+) - (β) - Fenebrene	9.483	0.59	9.480	0.80	9.470	0.51
2.	Germacrene-D	9.884	0.67	9.880	0.61	9.878	1.21
3.	*trans* –(β)-Caryophyllene	10.242	3.98	10.240	2.79	10.237	3.58
4.	β - Farnacene	10.385	1.07	10.376	0.67	10.379	1.02
5.	Caryophyllinoxide	12.086	1.60	12.082	1.23	12.078	1.88
6.	Precocene-II	12.882	63.05	12.853	57.08	12.886	51.70
7.	Precocene-I	13.164	13.27	13.149	1.10	13.158	16.41
8.	Hexadecanoic acid methyl ester	17.127	0.38	17.110	11.39	17.105	3.35
9.	α-linolinic acid	20.924	0.24	20.909	12.36	20.935	3.08
10.	Citrolinol	21.228	1.97	21.127	0.49	21.215	2.47
11.	*trans* - Squalene	38.104	13.18	38.089	11.48	38.106	15.48

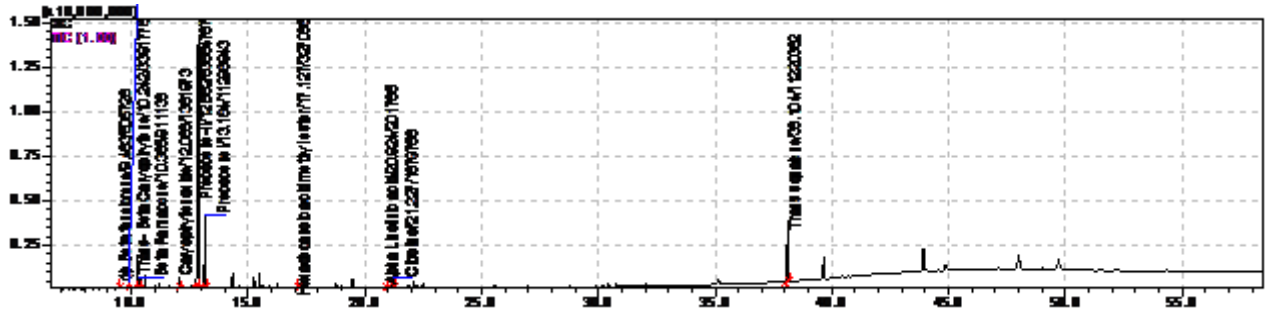


Fig. 2: GC-MS chromatogram of DCM Extract

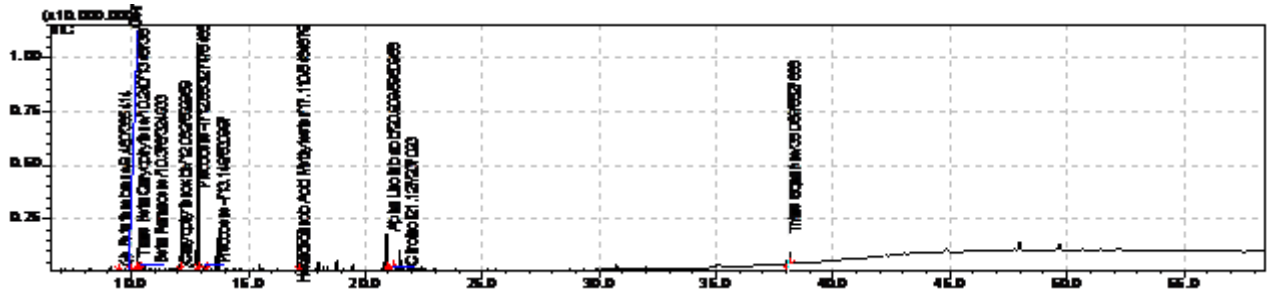


Fig. 3: GC-MS chromatogram of Methanolic Extract

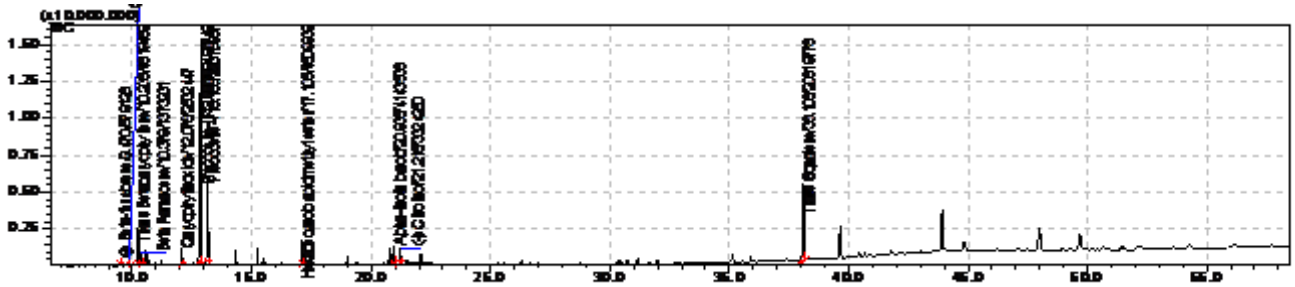


Fig. 4: GC-MS chromatogram of *n*-Hexane Extract

CONCLUSION

The comparative study of GC-MS shown that the concentration of phyto - constituents germacrene-D, caryophyllinoxide, precocene-I, citrolinol and *trans* - squalene in these three extracts are found in the order of *n*-hexane > DCM > methanol. The concentration of *trans* – (β) caryophyllene and β- farnacene are in order of DCM > Hexane > Methanol while the hexadecanoic acid methyl ester and α-linolinic acid is having more concentration in methanol as compared to *n* - hexane and DCM. It has been also analyzed that the concentration of (+) - (β) -fenebrene and precocene-II are found in the order of Methanol >DCM > Hexane and DCM > Methanol > Hexane, respectively.

It has been concluded from this study that quantitative comparative assessment of important phyto-constituents is useful for researchers to select a particular enriched extract for therapeutic efficacy.

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REFERENCES

1. Okunade AL. A review *Agratum conyzoides* L. (Asteraceae). Fitoterapia (ELSEVIER) 2002; 73: 1-16.
2. Calle J, Rivera A, Luis JG, Aguiar ZE, Niemeyer HM, Joseph NP. Insecticidal Activity of the Petrolium ether extract of *Ageratum Conyzoides* L. Revista Colombiana De Química (RCDQ) 1990; 19: 91-96.
3. Shekhar CT, Goyal A. A Comprehensive Review on *Ageratum conyzoides* L. (Goat weed). International Journal of Pharmaceutical and Phytopharmacological Research (IJPPR) 2012; 1(6): 391-395.
4. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman & Hall, London; 3rd Edition: 1998.
5. Kamboj A, Saluja AK., *Ageratum conyzoides* L.: A Review on its phytochemical and pharmacological profile. International Journal of Pharmacy & Pharmaceutical Sciences (IJPPS) 2011; 3(1): 94.
6. Awad NE, Kassem HA, Elkhayat ZA, El-feky AM, Matloub A A. Chemical composition and anti-inflammatory evaluation of *Ageratum conyzoides* L. leaves. Journal of Applied Sciences Research (JASR) 2013; 9: 21-26.
7. Padalia RC, Verma RS, Sundaresan V. Volatile Constituents of Three Invasive Weeds of Himalayan Region. Records of Natural Products (RNP) 2010; 4(2): 109-114.
8. Adjou, ES, Ahoussi ED, Degnon R., Soumanou MM, Sohounhloue, DCK., Investigations on bioactivity of essential oil of *Ageratum conyzoides* L., from benin against the growth of fungi and aflatoxin production. International Journal of Pharmaceutical Sciences Review and Research (IJPSRR) 2012; 13(1): 143-148.
9. Pertamawati & Nuralih. Chemical components and biological activities of volatile oil of *Ageratum conyzoides* L. The International Seminar on Chemistry 2008; 586-588.
10. Usman LA, Zubair MF, Olawore NO, M' Civer FA, Ismaeel RO. Chemical Constituents of flower essential oil of *Ageratum conyzoides* growing in Nigeria Elixir Org. Chem. (EOC) 2013; 54: 12463-12465