CHEMICAL COMPOSITION OF VOLATILE EXTRACT OF *ERIGERON BONARIENSIS*AND ITS ANTIMICROBIAL ACTIVITY

PoonamKushwaha, Darshan Singh* and Ganga Bisht

Department of Chemistry

Kumaun University, Nainital, India.

Corresponding author: Darshan Singh

1. Introduction

The genus *Erigeron* (family Asteraceae) consists of more than 400 species. A large number of the Erigeron species yield essential oils rich in biologically active polyacetylenic compounds /terpenoids and are reported to possess diverse biological activity, *viz.*, antibacterial, antifungal and genotoxic[1] (Awen et al., 2010).*Erigeron bonariensis* (L.) is Commonly called "Gulava" or "Mrichbooti".

ERIGERON BONARIENSIS

Erigeron bonariensis (synonym *Conyzabonariensis*)is a species of *Erigeron*, found throughout the tropics and subtropics as a pioneer plant; its precise native origin is unknown, but most likely Central America or South America. Common names include Flax-leaf fleabane, Wavy-leaf fleabane and Argentine fleabane[2, 3].It is a rare alien in south eastern England, found beneath walls and in cracks in pavements and concrete

driveways. It flowers in August and continues fruiting until the first frosts (sometimes as late as up to late December). It is instantly recognisable by its blue-green foliage, very narrow, undulate leaves, and purple-tipped involucral bracts.*E. bonariensis* grows up to 75cm in height and its leaves are covered with stiff hairs, including long hairs near the apex of the bracts. It can easily be confused with *Conyzacanadensis*, which grows taller[2, 3].

2. Medicinal Uses

It is traditionally used in urine problems and as tonic and astringent[4]. It is claimed to be used in diarrhoea, diabetes, scalding unine and hemorrhage of bowel, uterus and of wounds. It is also being used for babies who hesitates to suckle milk of their mother[4].

3. Previous Reports

The major constituents of *Erigeron* species include monoterpenes, sesquiterpenes, polyacetylenic compounds, diterpenoids and bioactive pyrone derivatives.Chemical composition of essential oils extracted by traditionally hydrodistillation (HD) technique from leaves, stems, flower heads and roots of *E. bonariensis* L. collected at different stages (spring, summer and autumn) have been previously investigated[5] (Mabrouk et al., 2011). Summer samples were rich in matricaria ester (1.6 to 76.4%) and caryophyllene oxide (1.6 to 22.6%). According to the literature, ecological factors of the biotope can affect growth as well as the quality and quantity of generated metabolites and the extraction techniques can affect the quality of the essential oil and its medicinal properties.Benzarti et al. reported caryophyllene oxide (18.7%), spathulenol (18.6%) and α-curcumene (10.2%) whereas Sardinian essential oil was richer in *cis* -lanchnophyllum ester (14.2%) and (E)-β-farnesene (12.0%) in Tunisian *E. bonariensis* obtained by traditional hydrodistillation[6].Quercetin and quercitrin were also being identified from the ether and ethyl acetate soluble fraction of *E. bonariensis*[7].

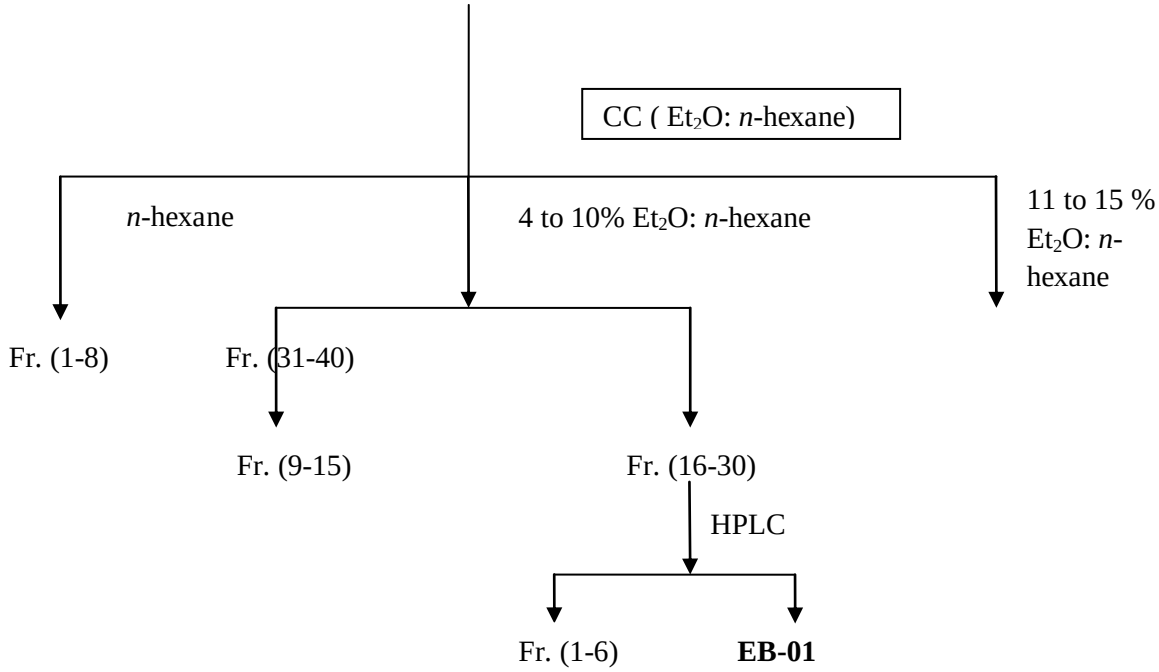
4. Plant collection and extraction

The plant material was collected from Dineshpur (District: Udham Singh Nagar) and identified from Botanical survey of India, Dehradun and Department of Botany, DSB Campus, Kumaun University, Nainital. The specimen herbarium has been deposited in Botanical survey of India, Dehradun and Department of Botany, DSB Campus, Kumaun University, Nainital. The fresh aerial parts (2 kg) of the plant material

were subjected to steam distillation. The distillates were saturated with NaCl and extracted with n-hexane and dichloromethane. The organic phase was dried over anhydrous Na2SO4 and the solvent was distilled off in rotary vacuum evaporator at 30°C.

Isolation and characterization of the major compound

Flow Sheet
***Erigeron bonariensis* (Essential oil)**



Physicochemical data of EB-01:

Yield: 150 mg, viscous liquid.

IR$^{KBr}\nu_{max}$cm^{-1}: 3065, 2927, 1634, 1454, 1376, 1070.

EIMS: 220 [M^+, $C_{15}H_{24}O$], 202, 187, 161, 159, 131, 119, 107, 105, 91(100%), 79, 77, 67, 55, 51, 41.

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: δ 4.95 (*s*, 2H), 4.85 (*s*, 2H), 2.83 (*dd, J* = 4.6 Hz), 2.61 (*q, J* = 9.3 Hz), 2.33 (*ddd, J* = 4.6, 7.7, 12.4 Hz), 2.23 (*ddt, J* = 4.6, 7.7, 9.3), 2.10 (*m*, 2H), 1.73 (*t, J* = 9.3 Hz), 1.62 (*m*, 3H), 1.42 (*m*,), 1.31 (*m*, 1H), 1.22 (*s*, 3H), 1.00 (*s*, 3H), 0.98 (*m*, 1H), 0.95 (*s*, 3H).

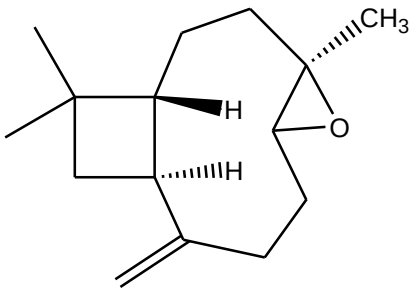
^{13}C-NMR (300 MHz, $CDCl_3$)δ ppm:δ 151.5, 112.5, 63.7, 59.8, 50.7, 48.7, 39.7, 39.1, 34.0, 29.9, 29.7, 27.3, 27.2, 21.6, 17.0.

Characterization of EB-01:

The compound EB-01 was obtained as colorless viscous liquid. The mass spectrum (EI) of the compound displayed a molecular ion peak at *m/z* 220, corresponding to the molecular formula $C_{15}H_{24}O$.

The presence of one exocyclic double bond was confirmed by two singlets at δ 4.85 and 4.97 ppm, supported by appearance of two signals at δ 151.8 and 112.7 in ^{13}C-NMR. The ^{1}H-NMR showed 2singlets at δ 0.98 and 1.00 for a *gem*-dimethyl group with attachment to the quaternary carbon.

The ^{13}C-NMR of the compound showed total of fifteen signals for 15 carbons in the molecule and the multiplicity assignments of the signals showed the presence of 3 methyl groups, 6 methylene, 3methine and 3 quaternary carbons. On the basis of these spectral data EB-01 has been identified as caryophyllene oxide. Finally, its identity was confirmed by comparison of its spectral data with literature reports[8].



caryophyllene oxide

6. Results and discussion

6.1. Terpenoid composition

The quantitative and qualitative analysis of the oil by GC and GC-MS, led to the identification of a total of 20 constituents, accounting 95.23 % of the total oil. The identified compounds are listed in Table 1 in order of their elution in Omega wax SP-2560 column (Figure 3). The oil was mainly dominated by two oxygenated sesquiterpenes*viz.*β-eudesmol (40.62 %) and caryophyllene oxide (34.13 %). The other volatile constituents of the essential oil werecarvacrol (8.94 %), γ-gurjunene (4.27 %)

and β-cedrene (2.25%).The structures of the major compounds have been shown in Figure3.1. In earlier reports *E. bonariensis* major compounds were matricaria ester or caryophyllene oxide and spethulenol or *cis*-lanchnophyllum ester, while in this study the results were in contrast to the previous reports because the the major compound was β-eudesmol (40.62 %) along withcaryophyllene oxide (34.13 %). This shows that the studied *E. bonariensis*from Uttarakhand India is chemically different from the previously reported *E. bonariensis.*

6.3. Antimicrobial activity

In this study, five microbial species were used to determine the possible antimicrobial activity of the oil and shown in Table 2. *Salmonella enterica* (formerly *Salmonella choleraesuis*) is a rod-shaped, flagellated, facultative anaerobic, Gram-negative bacterium and a member of the genus Salmonella and a number of its serovars are serious human pathogens. Most cases of salmonellosis are caused by food infected with *S. enterica*, which often infects cattle and poultry, though also other animals such as domestic cats[11]. Interestingly, the oil showed good activity against *Salmonella enterica* with ZOI 10.00 ± 0.00 mm and 50 µL/ mL MIC value. *Pseudomonas aeruginosa* is a common Gram-negative bacterium that can cause disease in animals, including humans. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in hypoxic atmospheres, and has, thus, colonized many natural and artificial environments. It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonizations occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal.Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment, including catheters, causing cross-infections in hospitals and clinics[9].The Essential oil was found to be moderately active against *Pseudomonas aeruginosa*having ZOI 8.33 ± 0.58 mm and 50 µL/ mL MIC values. *Escherichia coli*, belonging to the normal flora of humans, is an enterohemmoragic bacterium causes serious cases of food poisoning and preservatives to eliminate its growth are needed. The oil showed less antimicrobial activity then the

standards used against *E. coli* with 8.00±0.00 mm ZOI and 50 µL/ mL MIC value showing less potent activity against this bacterial strain. The essential oil wasless activity against *S. aureus*(ZOI 9.67 ± 0.58 mm and 100 µL/ mL MIC value) and*K. pneumoniae*(ZOI 6.77 ± 0.00 mm and 200 µL/ mL MIC).Gentamycin (30 µg/disc) and Kanamycin (30µg/disc) were used as positive controls.

The antimicrobial activity of the oil has been compared with the standard antimicrobial compounds in Figure 2.

7. Conclusion

The oil was characterized by the dominant presence of β-eudesmol(40.62 %) and caryophyllene oxide (34.13 %).Caryophyllene oxide was isolated and purified by column chromatography using ether: hexane as solvent and have been characterized by its ^{1}H and ^{13}C NMR data. The studied plant ischemotaxonomically different from previous reports on *E. bonariensis*from different locations.This report is in contrast to the previous because there is no other report showing β-eudesmolas major constituents along with the presence of but presence of the caryophyllene oxide is comparable to the other reports.

Significant antibacterial activity of the essential oil against twobacterial speciesindicates good bioactive potential of the volatile oil. The oil was found to be significantly active for *Salmonella enterica,Pseudomonas aeruginosa*and*Escherichia coli*. The essential oil was comparatively less active for*Staphylococcusaureus*and *Klebsiellapneumoniae*. The essential can effectively be used as antimicrobial agent for *Salmonella enterica,Pseudomonas aeruginosa* and*Escherichia coli*.Antimicrobial activity of essential oils is difficult to correlate to a specific compound due to their complexity and variability. It has been mainly explained through C_{10} and C_{15}terpenes with aromatic rings and phenolic hydroxyl groups able to form hydrogen bonds with active site of target enzymes, although other active terpenes, as well as alcohols, aldehydes and esters and their synergic effects can contribute to the overall antimicrobial effect of essential oils[10]. Therefore, the antibacterial results observed in this investigation might be related to the presence of β-eudesmol, caryophyllene oxide and carvacrol, although the synergistic effects of the diversity of major and minor constituents in the

essential oils should be taken into consideration to account for their overall biological activity.

Table 1.Chemical composition of *Erigeron bonariensis*essential oil.

S. No.	Compounds	RI	% Composition (FID)	Mode of Identification*
1.	α-pinene	932	0.48	a, b
2.	β-pinene	974	0.12	a, b
3.	α-terpenene	1014	0.09	a, b
4.	1,8-cineole	1026	0.06	a, b
5.	ocimene	1044	0.12	a, b
6.	linalool	1095	0.13	a, b
7.	1-terpeneol	1130	1.00	a, b, c
8.	borneol	1165	0.04	a, b
9.	terpenene-4-ol	1174	0.04	a, b
10.	piperitone	1249	0.33	a, b
11.	geranial	1264	0.62	a, b
12.	carvacrol	1298	**8.94**	a, b, c
13.	α-gurjunene	1409	0.21	a, b
14.	β-caryophyllene	1417	1.23	a, b, c
15.	β-cedrene	1420	2.25	a, b
16.	γ-gurjunene	1475	**4.27**	a, b
17.	matricaria ester	1522	0.35	a, b
18.	caryophyllene oxide	1582	**34.13**	a, b, c, d
19.	β-eudesmol	1649	**40.62**	a, b, c
20.	patchouli alcohol	1656	0.20	a, b, c
	Total		95.23	

*Mode of identification: Retention Index (LRI, Based on homologous series of n-alkanes; C_8-C_{24}), coinjection with Standards/Peak enrichment with known oil constituents, t= trace (<0.1%); (-) = not detected, a = Retention Index (RI) on Rtx-5 capillary column; b = MS (GC/MS) comparison with NIST and WILLEY and literature (Adams, 2007); c= co-injection with the standard compound; d= NMR.

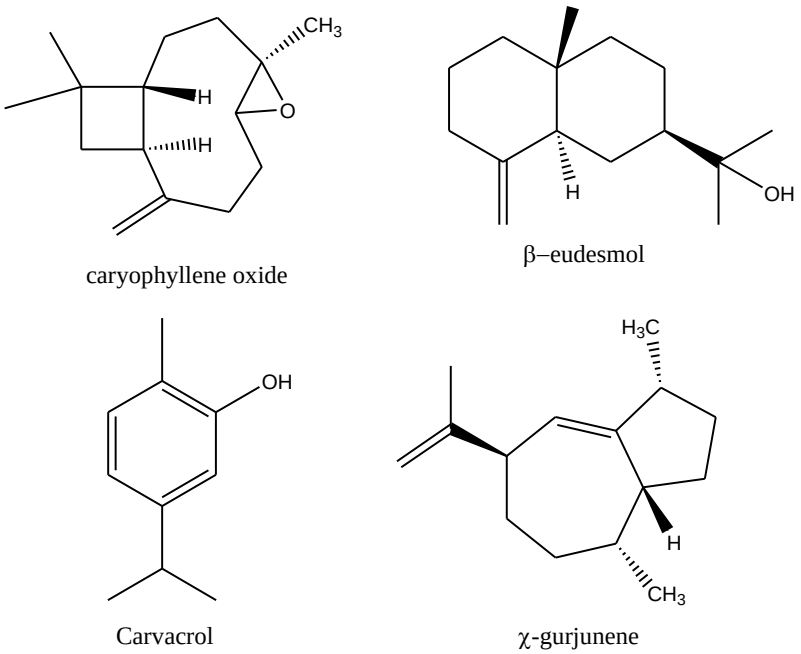


Figure 1.Structures of major compounds of *Erigeron bonariensis*essential oil.

Table 2.Antimicrobial activity of *Erigeron bonariensis*essential oil.

Bacterial strain	Essential oil		Standard antimicrobial			
			Gentamycin		**Kanamycin**	
	ZOI (mm)	**MIC** (µl/ml)	**ZOI** (mm)	**MIC** (µl/ml)	**ZOI** (mm)	**MIC** (µl/ml)
Klebsiellapneuminiae	**6.77±0.00**	**200**	10.67±0.58	50	10.67±0.58	50
Staphylococcusaureus	**9.67±0.58**	**100**	17.33±0.58	50	16.33±0.58	50
Escherichia coli	**8.0±0.00**	**50**	14.67±0.58	50	13.33±0.58	75
Salmonella enterica	**10.00±0.00**	**50**	10.67±0.58	50	9.67±0.58	100
Pseudomonas aeroginosa	**8.33±0.58**	**50**	13.33±0.58	50	12.67±0.58	50

*Zone of inhibition (ZOI) is given at 200 µl/ml. MIC= Minimum inhibitory concentration.

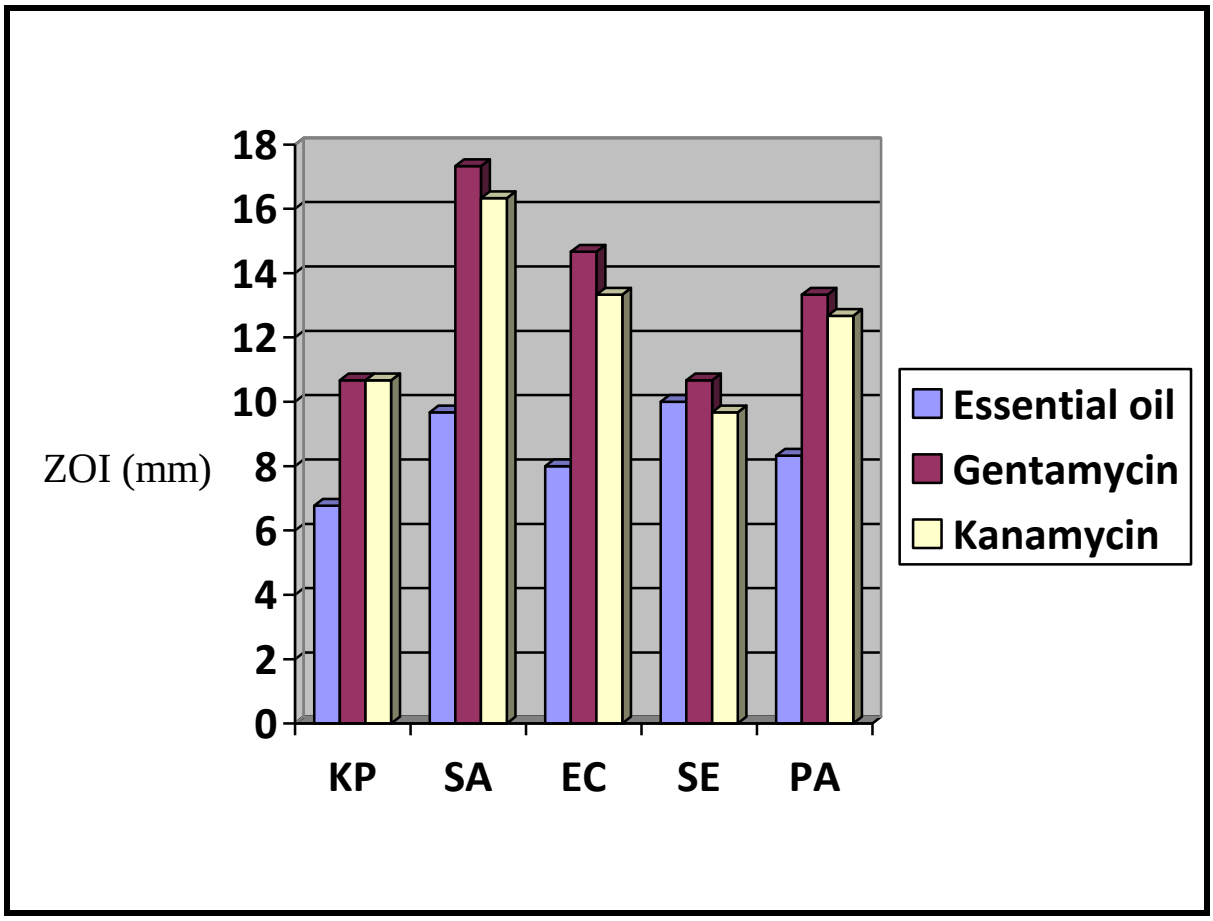


Figure 2.Comparative antimicrobial activity of the essential oil.

KP = *Klebsiellapneumoniae*
SA = *Staphylococcusaureus*
EC = *Escherichia coli*
SE = *Salmonella enterica*
PA = *Pseudomonas aeruginosa*

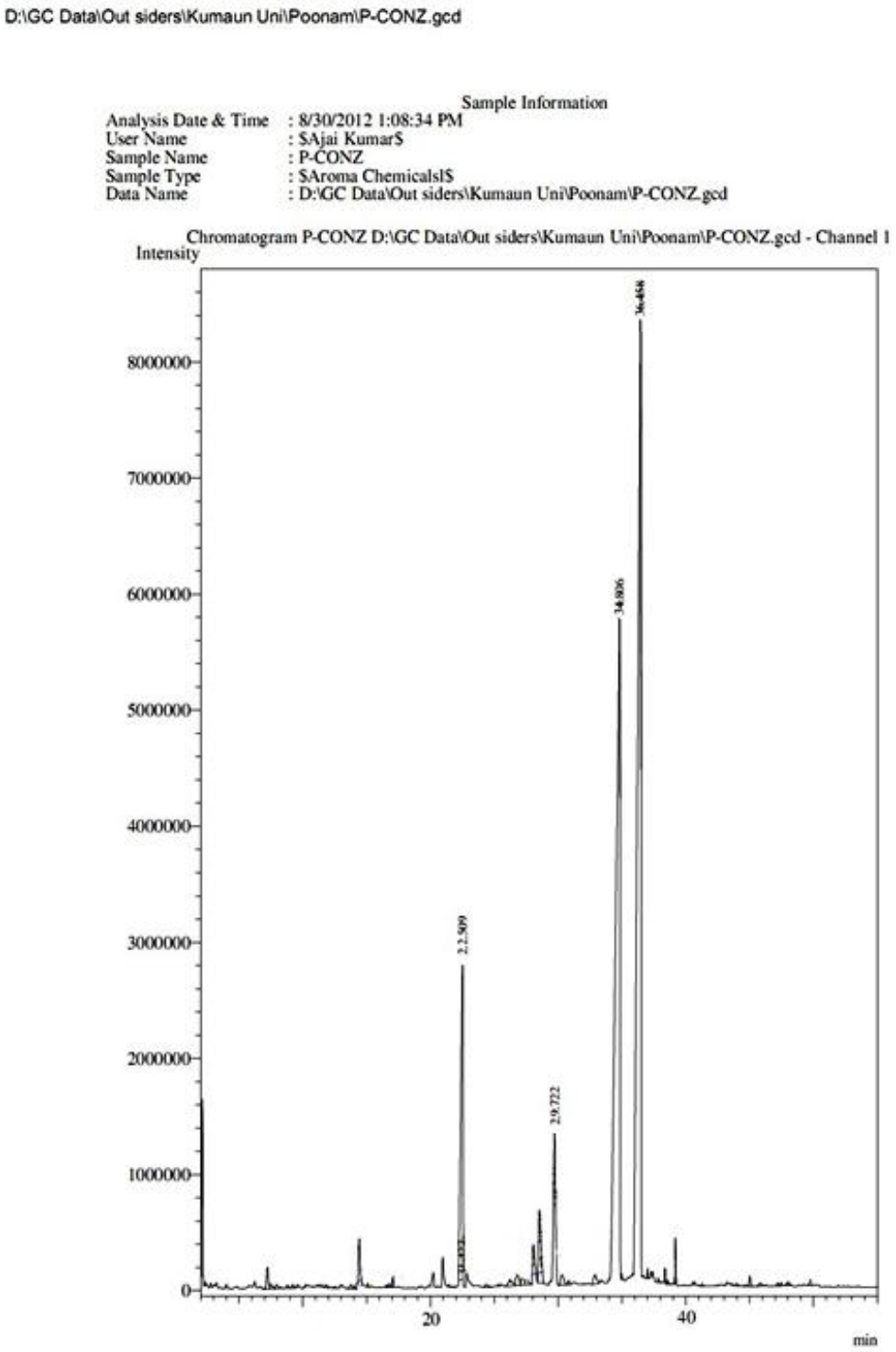


Figure 3.Gas chromatogram of *Erigeron bonariensis*essential oil.

8. References

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