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EXTRACTION AND PARTIAL PURIFICATION OF BETA-AMYRIN FROM CRUDE METHANOLIC LEAF EXTRACTS OF *ALOE VERA* L. FOR ITS TOPICAL ANTI-INFLAMMATORY ACTIVITY ON ALBINO MICE

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***ABSTRACT**: The present research work was undertaken to extract and isolate beta amyrin from Aloe vera L. which is one of the well-known medicinal plants in Ayurveda. The latex of this plant is commonly termed as Aloe, which shows prominent active ingredients in it. The topical application of aloe is well practised traditionally. The most important constituents of aloes are the two aloins, namely barbaloin and isobarbaloin with small portion of beta-amyrin (Oleanane skeleton). The main course and basic treatment line for inflammatory diseases remain one of the major health problems. Hence in present work isolated and partially purified beta amyrin gel was formulated and was employed to reduce induced inflammation topically in 1-4 hour time period. Spectral studies confirmed the presence of beta amyrin. Topical anti-inflammatory activity using mice paw confirms 54.59% activity with 2 mg/kg concentration.*

Key words: *Aloe vera L., Albino mice, Anti-inflammatory activity, beta-amyrin, Topical activity.*

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I INTRODUCTION

Aloe vera L. is one of the well-known medicinal plants in Ayurveda and other folk literates. Commonly *Aloe vera* L. is known as burn plant in English, Gheekumari in Hindi. This plant species fits in Asphodelaceae family. Nearly 400 species of aloe are well-known across world. All of them are perennial with fibrous roots and fleshy leaves grow well in hot, dry climate with standing, terminal spikes of yellow or purplish colour flowers. Peripheral bundle sheath cells in leaf possess yellow latex which strongly unpleasant in taste. This latex is commonly termed as Aloe [1]. However, topical application of aloe is very effective due presence of anthra-quinones and triterpenoid content. The most important constituents of aloes are the two aloins, namely barbaloin and isobarbaloin with small portion of beta-amyrin (Oleanane skeleton) [2]. Triterpins are secondary plant metabolites widespread in fruit peel, leaves and stem bark. In particular the beta-amyrin- oleanane display various pharmacological effects while being devoid of prominent toxicity**.** The main course and basic treatment line for inflammatory diseases remain one of the major health problems. Inflammation is a complex biological rejoinder of vascular tissue against injury which is characterized by redness, warmth, swelling and pain. Sustained effect of inflammation leads to serious health conditions like inflammatory bowel disease (IBD), degenerative diseases like rheumatoid arthritis, atherosclerosis, Alzheimer's, asthma and cancer. At present two drugs namely non-steroidal anti-inflammatory drugs (NSAIDs) and disease-modifying anti-rheumatic drugs (DMARDs) are used in the treatment of inflammatory diseases. But these drugs are well known for their side effects. Therefore it is very necessary to find new therapeutic agents for a variety of inflammatory diseases. Very few literatures were available regarding topical anti-inflammatory activity of beta amyrin using albino mice. Therefore present research work was carried out for extraction and isolation of beta amyrin using crude leaf extract of *Aloe vera* L.

II MATERIALS AND METHODS

For present research work healthy *Aloe vera* L. leaf material was made available from local parts of Solapur region. Later it was identified by the Botanist at D.B.F Dayanand College

of Arts and Science, Solapur. All reagents and glassware's were made available from biotechnology research laboratory from D.B.F Dayanand College of Arts and Science, Solapur and V.G Shivdare college of Arts, Commerce and Science, Solapur.

Preparation of methanolic extracts

The fresh leaves of *Aloe vera* L. were collected from Western parts of Maharashtra and plant material was authenticated by Botanist at department of Botany, D.B.F Dayanad college of Arts and Science, Solapur. The leaves were dried under shade and then powdered using electrical grinder. Thousand grams of powdered leaves were extracted with equivalent millilitres of methanol as a solvent by cold extraction method. The resulting extract was filtered through metal leaf filters. The filtrate was evaporated till sticky material was obtained.

Partial purification of crude extracts

Extracted plant crude extracts were partially purified using column chromatography with varying fractions of hexane acetone solvents. All collected fractions were re-purified using same technique, using methanol-chloroform fractionation [3].

Preparative Thin layer chromatography

Among five fractions, fraction A,B,C,D and E were re-crystalized using PTLC methodology given by Poul *et al*., [4].

Spectral analysis of crystalized fractions

Spectral tools like FT-IR (Thermo scientific Nicolet iS10), NMR (Buker) and GC-MS (Shimadzu HS-20) were used to find bio-actives in crystalized fractions [5-6]. Peak analysis was done by comparing with standard available drugs.

Formulation and preparation of topical gel

Gel base was prepared using 1% w/w carbopol-934 with 0.5 % xantham gum as a gelling agent in double distilled water using stirrer [7]. The pH of the gel was adjusted to neutral by addition of 1µg/ml triethanolamine. To this molten gel base, 2 mg/kg of partially purified beta amyrin dissolved in pure ethanol was added.

Selection and maintenance of animals

Healthy albino mice of either sex, weighing 20-25 gm, were used. All animals were housed as per guidelines of CPSEA at Aarya Biotech Pvt. Ltd, Dhule (M.S), India (Registration number 1822/PO/RcBiBt/S/15/CPCSEA) with IAEC approval (Approval number 7/AB/2017).

Evaluation of anti-inflammatory activity Animals

Carrageenan-induced mice paw oedema

For present research work all animal were gathered in three groups (Control, Test and Standard) of six animals each. In all group inflammation in mice left paw region was induced using 0.1 ml 1 % carrageenan using sterile needle.

Anti-inflammatory activity

Anti-inflammatory activity of experimental gel containing 2 mg/kg beta-amyrin, was applied as per method described by [7]. Control mice groups received plain gel base and 1% Diclofenac gel, applied in the same way was used as a standard. Paw volume was measured immediately after carrageenan injection and at 1- 4 hours of intervals using mercury displacement method [4].

Statistical Analysis

All recorded data were analysed as the mean ± SEM (Standard Error Mean) and by means of analysis of variance (ANOVA). Statistical significance was calculated at $p<0.05$.

III RESULTS AND DISCUSSION

Preparation of methanolic extracts

From 1 kg crude methanolic extracts about 10 gm/kg of dried sticky material was obtained in successive 7 days [3].

Partial purification of crude extracts

Colum chromatography

Using Hexane and Acetone fractionation technique five grades were obtained with retention time of 1 ml/min. All fractions were again purified using re-column. Fraction E showed great extent of crystallization pattern.

Preparative Thin layer chromatography

Preparative thin electrophoresis yielded a sharp but thin band of beta amyrin with other organic moieties. RF value of 0.69 was noted for extracts which correlates with the results obtained by [8].

Spectral analysis of crystalized fractions

Partially purified and recrystallized crude drug was subjected to spectral analysis. Obtained peeks were compared with available literatures as well as with peak libraries [9].

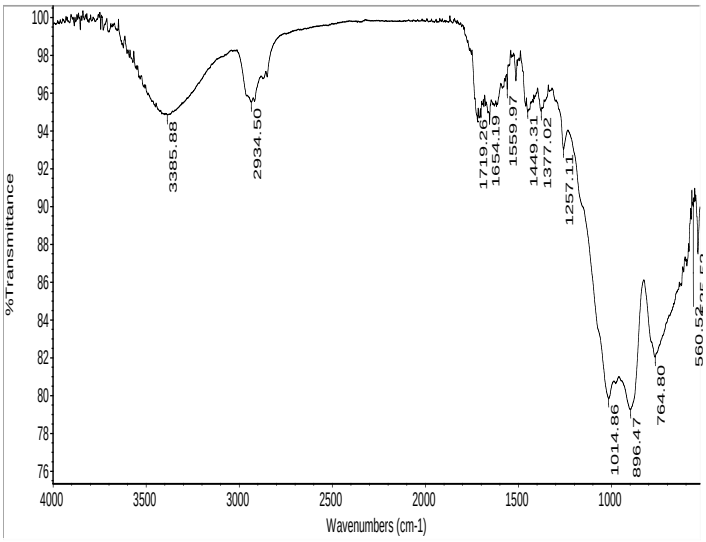


Figure 1. FT-IR analysis partially purified beta amyrin

Table 1. FT-IR functional group analysis of partially purified beta amyrin

Wavenumber cm $^{-1}$ Position	Intensity	Active group
535.52	87.560	Alkyl halide C-Br
560.52	89.983	Alkyl halide C-H
764.80	82.024	Benzene ring (meta substituted)
1014.86	79.805	Alkane
1257.11	93.011	Ether (aromatic)
1377.02	95.022	Alkane (methyl)
896.47	79.245	Alkene
1449.31	94.985	Alkane (Strong)
1559.97	96.190	Nitro (aromatic)
1654.19	94.489	Amine (primary)
2934.50	95.515	Amine (NH4 ion)
1719.26	94.480	Ketones (saturated)
3385.88	94.844	Amine (medium peak)

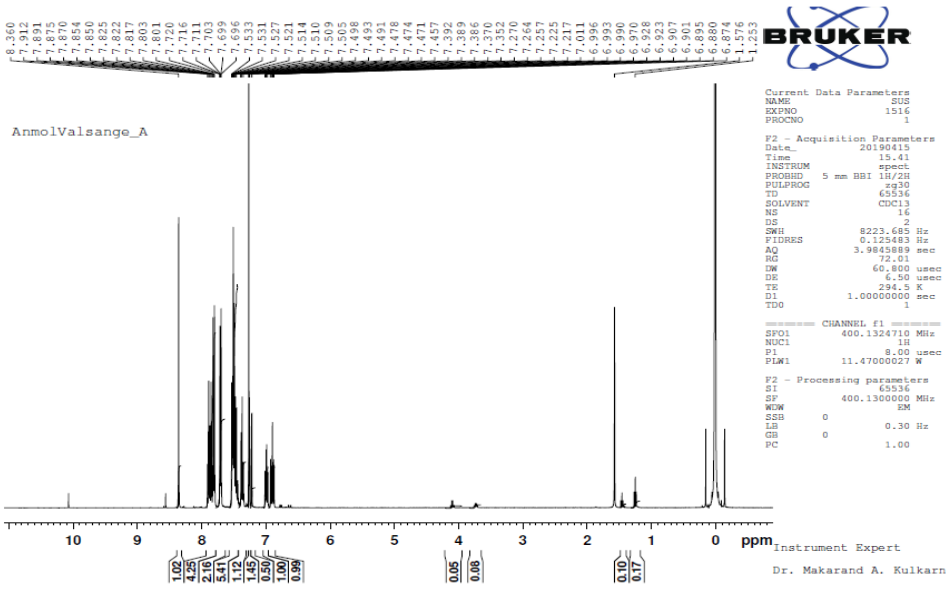


Figure 2. NMR peak analysis partially purified beta amyrin

GC-MS analysis of E fraction of *Aloe vera* L. leaf extract shwed presence of 4-(2, 3-Dihydroxy-3methyl butoxy) furo (3, 2-g) chromen-7-one which occupied 7.38 % area on chromatogram, similarly 1-Butanol, 3-methyl, acetate occupied 9.27 % area, Octanal, 7-hydroxy-3, 7-dimethyl with area of 12.18 %. The major area on chromatogram was occupied by Beta –Amyrin with 28.77 % as shown in figure **3, [10].**

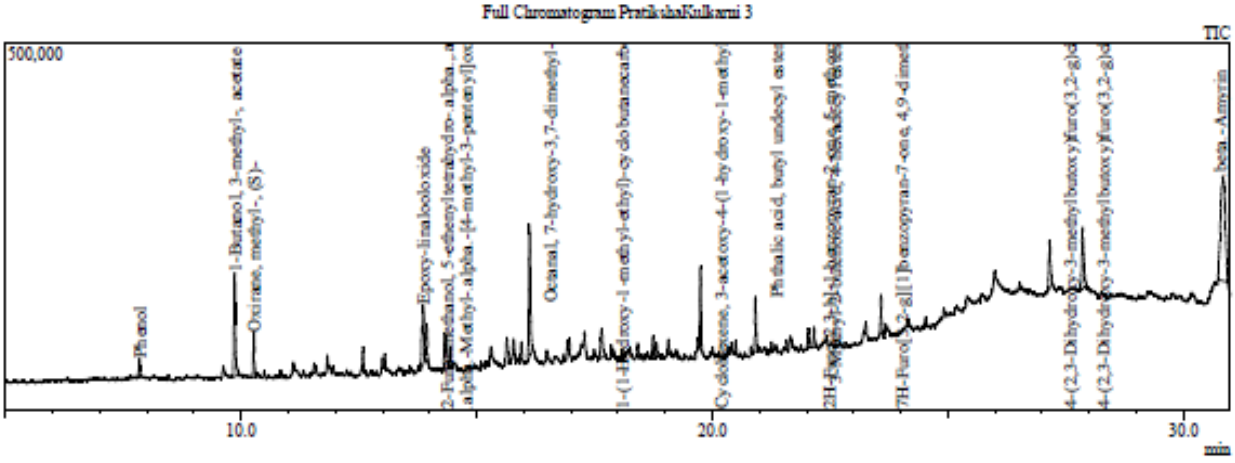
Punyashlok Ahilyadevi Holkar Solapur University

Sample Information

Analyzed by	: Dr.Makarand Kulkarni
Analyzed	: 7/2/2019 1:35:15 PM
Sample Name	: PratikshaKulkarni 3
Sample ID	: PratikshaKulkarni 3
Vial #	: 7
Injection Volume	: 4.00 mL HS
Data File	: D:\GCMS\Results\PratikshaKulkarni 3.qgd
Method File	: D:\GCMS\METHOD\General Autosampler method.qgm
Tuning File	: D:\GCMS\Tuning file\autosampler02072019.qgt

(Lemon Extract!=)[Comment]

Full Chromatogram PratikshaKulkarni 3



Peak Report TIC

Peak#	R.Time	Area	Area%	Name	Similarity
1	7.843	39252	1.11	Phenol	84
2	9.863	328296	9.27	1-Butanol, 3-methyl-, acetate	86
3	10.271	111869	3.16	Oxirane, methyl-, (S)-	89
4	13.871	220877	6.23	Epoxy-linalooloxide	79
5	13.939	112129	3.16	2-Furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-, cis-	78
6	14.322	174086	4.91	.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol	88
7	16.125	431691	12.18	Octanal, 7-hydroxy-3,7-dimethyl-	86
8	17.657	126795	3.58	1-(1-Hydroxy-1-methyl-ethyl)-cyclobutanecarboxylic acid	81
9	19.752	189333	5.34	Cyclohexane, 3-acetoxy-4-(1-hydroxy-1-methylethyl)-1-methyl-	72
10	20.919	147721	4.17	Phthalic acid, butyl undecyl ester	80
11	22.047	61734	1.74	2H-Furo[2,3-h]-1-benzopyran-2-one, 5-methoxy-	76
12	22.163	53100	1.50	3-Methyl-2-butenoic acid, 4-hexadecyl ester	82
13	23.584	93543	2.64	7H-Furo[3,2-g][1]benzopyran-7-one, 4,9-dimethoxy-	91
14	27.162	171908	4.85	4-(2,3-Dihydroxy-3-methylbutoxy)furo(3,2-g)chromen-7-one	88
15	27.863	261289	7.38	4-(2,3-Dihydroxy-3-methylbutoxy)furo(3,2-g)chromen-7-one	94
16	30.836	1019255	28.77	.beta.-Amyrin	92
		3542878	100.00		

Figure 3. GC-MS peak analysis crude extract

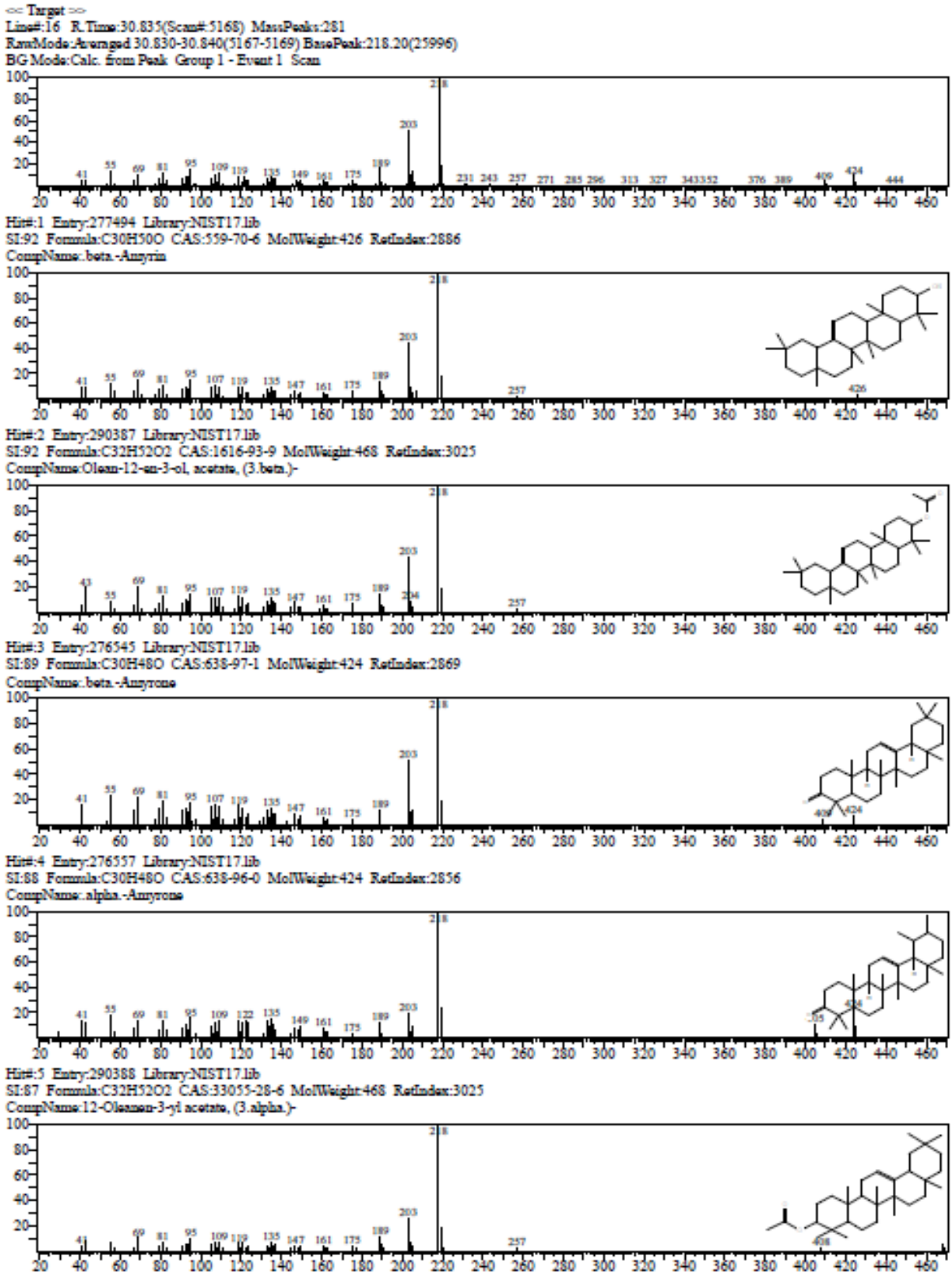


Figure 4. GC-MS Peak analysis of extracted beta amyrin.

Formulation and preparation of topical gel

Gel was successively formulated using active ingredients as discussed by [11]. Prepared gel was compared with standard commercially available gel (Table 1, 2). There was no change in colour observed even after 3 months of preparation. The prepared herbal gel was evaluated to various parameters. The gel was greenish brown in colour with a glowing form and cooling sensation throughout the evaluation period [12]. The pH was constant throughout the study to about 6.9. The stability study's results exposed the preparation was stable at normal storage conditions, as shown in table 1.

Evaluation of anti-inflammatory activity animals

Carrageenan-induced mice paw oedema

Inflammatory activity of 1 % (0.1 ml) carrageenan was recorded using mercury displacement technique. The initial mercury displacement of 0.126 ± 0.0042 mm showed no inflammation (normal paw), whereas mean value of mercury displacement 0.303± 0.0021 showed 100 % inflammation after 1/h hour. However there was no significant reduction in inflammation was noted even after 4 hours, therefore group I was used as positive control during study. Group II animals were treated in same manner but with only plain gel base i.e. negative control whereas group III was treated with experimental gel base, which is test group.

Anti-inflammatory activity of beta amyrin (formulated topical gel)

Table 2 shows anti-inflammatory activity beta amyrin formulated gel in comparison with positive and negative group. Alone 1 % diclofenac gel showed 69.02% activity whereas no inflammation reduction has been noted with plain gel and 1 % carrageenan. Group III *i.e*. experimental group showed 54.59 % activity.

Table 2. Anti-inflammatory activity beta amyrin formulated topical gel on albino mice left paw after 0.1 ml (1 %) carrageenan administration (Mean ± S.E.M)

Animal Group	Mercury displacement (ml)/Hour (Average volume Mean ± S.E.M)									Average % Inhibition after 4 hour
	Initial hour	½ hour	1 hour	1 ½ hour	2 hour	2 ½ hour	3 hour	3 ½ hour	4 hour	
Group I	0.126 ± 0.0036	0.301 ± 0.0021	0.302 ± 0.0016	0.299 ± 0.0016	0.297 ± 0.0021	0.295 ± 0.0022	0.293 ± 0.0021	0.289 ± 0.0022	0.289 ± 0.0030	0
Group II	0.129 ± 0.0036	0.303 ± 0.0021	0.302 ± 0.0021	0.299 ± 0.0016	0.289 ± 0.0016	0.296 ± 0.0025	0.236 ± 0.0033	0.289 ± 0.0047	0.288 ± 0.0021	0
Group III	0.126 ± 0.0034	0.299 ± 0.0016	0.289 ± 0.0034	0.285 ± 0.0034	0.258 ± 0.0047	0.241 ± 0.0047	0.234 ± 0.0047	0.193 ± 0.0047	0.189 ± 0.0034	69.02
Group IV	0.126 ± 0.0014	0.300 ± 0.0022	0.302 ± 0.0022	0.298 ± 0.0029	0.263 ± 0.0024	0.277 ± 0.0019	0.244 ± 0.0023	0.256 ± 0.0019	0.198 ± 0.0025	54.59

All results were analysed using one way ANOVA, by calculating f values. At present f value obtained was 5.30 which is significant at $p<0.05$ [13].

IV CONCLUSION

Besides concentration, *Aloe vera* L. proves to be good source for the isolation of beta amyrin. From results and analysed data, beta amyrin proves to be good and new alternative in the management of inflammation externally. Although further purification and recrystallization may improves quality in research.

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