3

Isolation and characterization of the Non-polar phytocomponents of *Aloe vera* gel and evaluation of the pharmacokinetics, distribution and pharmacodynamics in rats

3.1. Introduction

Aloe vera, a succulent perennial herb belonging to family Liliaceae, widely distributed in the tropical and subtropical regions of the world has been chosen in the present study. The name *Aloe* comes from the Greek word referring to the bitter juice from the plant's leaves, though it is said to be derived from the word alloeh in Arabic or allal in Hebrew, both meaning shining bitter substance. Plants of the genus *Aloe* have perhaps the longest recorded history of medicinal usage and are amongst the most widely used plants for traditional medicinal purposes worldwide.

3.1.1. Classification of *Aloe vera*

Kingdom	Plantae
Division	Angiospermae
Order	Asparagales
Family	Asphodelaceae/ Liliaceae
Genus	*Aloe*
Species	*Aloe vera*

3.1.2. Vernacular names of *Aloe vera*

Common name: *Aloe vera*; Hindi: Gheekumari, Gwarpaathu; Bengali: Ghrita Kumari; Gujarati: Kunvarpaathu; Marathi: Khorpad; Tamil: Kathalai; Malayalam: Kattar vazha, Cherukattazha; Nepali: Ghyu Kumaari; Telugu: Kalabanda; Assamese: Chaal Kunwari; Kannada: Lolesara.

3.1.3. Part of use: Gel isolated from the leaves

3.1.4. Distribution of *Aloe vera*

Aloe vera is said to have originated in Sudan, after which it spread throughout the Mediterranean region and most other warm regions of the world (Grindlay and Reynolds 1986). Most widely spread in Asia, South America, Africa and Southern Europe, the plant can grow almost anywhere in the world, because it is not pretentious, it doesn't require special conditions for cultivation, as well it can receive the necessary nutritional substances and moisture even from the air.

3.1.5. Morphology of *Aloe vera*

The leaves of the *Aloe* plant grow from the base in the rosette pattern. Mature plants can grow as tall as 2-4 feet long and a base of 3 or more inches in diameter. Each plant usually has 12 - 16 dagger- shaped leaves that are green to grey-green in colour and decorated with whitish spots on both foliar surfaces, fleshy, tapering, spiny marginated and filled with a clear viscous gel. It is a stem less or short stemmed plant. The tubular flowers are orange and densely clustered at the stem apex; corolla is yellowish, tubular and up to 2.5 ± 0.5cm in length. The seeds are winged and held in dry capsules and doesn't have calyx. Fruits of aloe are triangular capsules. Roots grow wide and not too deep in soil. Each leaf is composed of three layers. An inner clear gel that contains 99% water and rest is made of glucomannans, amino acids, lipids, sterols and vitamins. The middle layer of latex is the bitter yellow sap and contains anthraquinones and glycosides. The outer layer is thick and it is of 15 - 20 cells called as ring and has protective function which synthesizes carbohydrates and proteins.

3.1.6. Traditional Uses of *Aloe vera*

Aloe vera is known in Ayurveda as kumari, which means "beautiful young girl." It was first used to treat illnesses that affect young females, such as acne and menstruation difficulties. *Aloe vera* gel has a wide variety of effects on the human body, according to the ancient Ayurvedic scriptures (Nadkarni, 1976; Risvan et al., 2017). In Western society, *Aloe vera* is one of the few herbal medicines in common usage, and it has found widespread use in the cosmetic, pharmaceutical, and food industries (Foster et al., 2011). *Aloe* leaves secrete two different types of exudates. One is a bitter reddish-yellow juice found in the pericyclic cells underneath the leaves' highly cutinized epidermis. This "juice" has traditionally been used as a laxative and in dried form. The presence of aloin, aloe-emodin, and similar chemicals gives it a bitter taste. The thin-walled tubular cells in the leaf's inner core zone (parenchyma) generate

a clear, slippery mucilage or gel as the other exudate. The pharmacological active components are concentrated in the gel and rind of *Aloe vera* leaves, according to clinical studies. Burns, allergic reactions, rheumatoid arthritis, rheumatic fever, acid indigestion, ulcers, diabetes, skin diseases, dysentery, diarrhoea, piles, and inflammatory conditions of the digestive system and other internal organs, such as the stomach, small intestine, liver, kidney, and pancreas, are just a few of the conditions that bioactive compounds from *Aloe vera* can help with (Joseph and Raj, 2010). The active ingredients have been shown to have analgesic, anti-inflammatory, antioxidant and anticancer agent. Today, it has a strong global reputation due to its widespread usage in cosmetics, particularly to treat burns and sunburn, to aid wound healing, and to fight against cell aging. *Aloe vera* plant also used as reinforce the immune system and improve blood circulation.

3.1.7. Phytochemicals present in *Aloe barbadensis* Miller:

Despite their wide acceptance, studies from different laboratories often report wide variations in the therapeutic bioactivities from within the same *Aloe* species, even when the same extraction procedures are used. Furthermore, leaves from individual *Aloe* plants within the same species may have widely varying levels of the bioactive phytochemicals. Phytochemical analyses have shown that many *Aloe* species contain various carbohydrate polymers (notably glucomannans) and a range of other low molecular weight phenolic compounds including alkaloids, anthraquinones, anthrones, benzene and furan derivatives, chromones, coumarins, flavonoids, phytosterols, pyrans and pyrones (Cock 2015). *Aloe barbadensis* is recognized for possessing fatty acids, phytosterols including lupeol, Cholesterol, Campesterol, beta-Sitosterol; antioxidant vitamins, including vitamin B12, folic acid, vitamin A, vitamin C and choline; hormones consisting of auxins and gibberellins; enzymes, consisting of alliinase, lipase, bradykinase, amylase, carboxypeptidase, catalase, cellulase, and peroxidase ; minerals such as copper, calcium, chromium, zinc, sodium, potassium, magnesium, and manganese; and finally glucose and fructose, polysaccharides forming sugars within the plant (Radha and Laxmipriya, 2015; Sharma et al., 2014; Zhang et al., 2018). The variation in concentration of these chemical constituents is based on the plant part used, extraction process, solvent, stage of growth, and plant source. It has been well established through various reports that the medicinal properties of plants can be attributed to wide array of phytoconstituents present in them which in turn can be held responsible for their physiological action on human body (Arya and Parmar, 2020; Hasan, et al., 2009). The previous literature and our studies have already demonstrated the anti-

hyperglycaemic (Tanaka et al., 2006), anti-dyslipidemic (Misawa et al., 2008; 2012; Desai et al., 2012) and ovarian structure-function modulatory property (Radha and Laxmipriya, 2016) of phytosterols present in *Aloe vera* gel. These reports indicate that the phytosterols and other non-polar phytochemicals present in *Aloe vera* gel have a potential to manage metabolic syndromes like diabetes, cardiovascular diseases and PCOS by interfering with the cholesterol absorption and metabolism as well as steroid hormone synthesis (Moghadasian and Frohlich, 1999).

It is a well-known fact that herbal medicines are a complex mixture of a myriad range of phytochemicals working together in a synergistic way to bring about the desired therapeutic effect. But the presence of such complexity also increases the chances of interactions (Meshesha, et al., 2020). Hence, chemo profiling and marker analysis becomes one of the most vital parameters in the standardization of a polyherbal formulation (Mukherjee, 2019). In order to understand the different interactions and toxicity within a biological system, it is utmost important to evaluate the bioavailability, pharmacokinetic and pharmacodynamic properties of the drug candidates. Surprisingly, only a few recent studies have focused on the isolation of the phytochemicals from *Aloe vera* (Tanaka et al., 2006) and understanding their bioavailability and pharmacokinetics (Dong et al., 2020; Yu et al., 2016; Dey et al., 2017), despite the profound usage of *Aloe vera* plant for cosmetic, dietary and therapeutic purposes. Performing such studies could facilitate optimization of drug candidates' absorption, distribution, metabolism, excretion and toxicity properties, with the ultimate goal of developing a drug with an adequate concentration-time profile in the body for the desired efficacy and safety profile. Understanding of the above said parameters are very important for translational research.

With this background, the present chapter aimed to isolate and characterize the non-polar phytochemicals from *Aloe vera* gel and also explored their pharmacokinetic and pharmacodynamic properties in adult female rats.

3.2. Materials and methods

Broadly, the methods can be categorized into two major parts. The first part of the chapter deals with the isolation of non-polar phytochemicals by column chromatography, its characterization by chromatographic techniques like Thin Layer Chromatography (TLC), Gas Chromatography- Mass spectrometry (GC/MS) and High-performance liquid chromatography (HPLC). The detailed methodology employed in this study have been discussed in detail in

Chapter 2. Following which, we performed the pharmacokinetic, tissue distribution, excretion and toxicity analysis in adult female Charles Foster rats. All the methodologies followed for this study has been described earlier in Chapter 2.

3.3. Results

3.3.1. Phytochemical Analysis

The yield and nature of the non-polar extracts of *Aloe vera* gel prepared by overnight shaking method is given is Table 3.1. It can be observed that as the polarity of the solvent increases, it resulted in high extract yield. However, phytochemical screening of the extracts demonstrated that Petroleum ether extract of *Aloe vera* gel was rich in phytosterols, glycosides, terpenoids and carbohydrates as compared to other non-polar extracts (Table 3.2). Hence, further studies were conducted using petroleum ether extract of *Aloe vera* gel. As clear from Table 3.1, the yields of the extracts prepared by overnight shaking method were very low, hence, we opted for Soxhlet extraction method. Details of which has been provided in Chapter 2. Data from Table 3.3 demonstrates that there has been a significant improvement in the yield of the extract after performing Soxhlet extraction procedure. The concentration of total phytosterols present in the petroleum ether extract of *Aloe vera* gel was estimated to be 624 $\pm$ 32.83 µg/g.

Table 3.1: %Yield and nature of the non-polar extracts of *Aloe vera* gel obtained after overnight shaking method

	Hexane	**Petroleum Ether**	**Toluene**	**Chloroform**	**Ethyl Acetate**
Dielectric Constant	1.88	2.0	2.38	4.81	6.02
% Yield dry weight (mg)	0.12	0.35	0.59	0.61	1.07
Colour	Faint yellow	Yellow	Yellow	Buff	Faint yellow
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Consistency	Sticky	Sticky	Sticky	Sticky	Solid

Table 3.2: Phytochemical Screening of non-polar extracts of *Aloe vera* gel obtained after overnight shaking method

	Hexane	Petroleum Ether	Toulene	Chloroform	Ethyl Acetate
Phytosterols	+	+++	+	+	++
Glycosides	+	++	-	-	+
Terpenoids	+	++	+	+	+
Flavonoids	-	-	-	-	+
Alkaloids	-	-	-	-	-
Tannins	-	-	-	-	-
Phenols	-	-	-	-	-
Saponins	-	-	-	-	-
Quinones	-	-	-	-	-
Proteins	-	-	-	-	-
Carbohydrates	-	++	+	+	+

Table 3.3: %Yield, nature and quantitative estimation phytosterols in the petroleum ether extract of *Aloe vera* gel obtained after Soxhlet extraction method

	% Yield dry weight (mg)	Colour	Odour	Consistency	Phytosterol Content (µg/g)
Petroleum ether extract of *Aloe vera* gel	4.95%	Dark greenish yellow	Characteristic	Sticky	624 $\pm$ 32.83

3.3.2. Isolation and Characterization of Non-polar Phytochemicals from *Aloe vera* gel

Column chromatography was performed as described in Chapter 2. The completion of elution of the component(s) was confirmed when evaporating a small fraction of eluent left no residue. The overall summary of column elution and the compounds isolated is presented in Table 3.4. To ensure eluent homogeneity, the eluents were analysed using pre-coated Thin Layer Chromatography (TLC) plates with appropriate mobile phase. After TLC of all the fractions, fraction no. 11 to 18 (LP1), fraction no. 29 to 34 (LP2), fraction no.59 to 65 (LP3), fraction no.

69-75 (LP4) and fraction no. 95-99 (LP5) were selected for further analysis. The % yield and nature of the isolates are given in Table 3.4

Table 3.4: Summary of fractionation of Petroleum ether extract of *Aloe vera* gel by column chromatography

Fraction No.	**Eluting Solvent**	**Compound (s) isolated**	**% Yield w/w**
1-6	Benzene	-	
7-10	Benzene: Diethyl ether (95:5)	-	
11-18	Benzene: Diethyl ether (90:10)	LP1 (creamish white colored, sticky and odorless)	0.09
19- 23	Benzene: Diethyl ether (85:15)	-	
24- 28	Benzene: Diethyl ether (80:20)	-	
29- 34	Benzene: Diethyl ether (75:25)	LP2 (brown colored, sticky and odorless)	0.058
35- 41	Benzene: Diethyl ether (70:30)	-	
42- 45	Benzene: Diethyl ether (60:40)	-	
46-52	Benzene: Diethyl ether (50:50)	-	
53-58	Benzene: Diethyl ether (45:55)	-	
59-65	Benzene: Diethyl ether (40:60)	LP3 (white colored, powdered and odorless)	0.076
66-68	Benzene: Diethyl ether (30:70)	-	
69-75	Benzene: Diethyl ether (20:80)	LP4 (creamish white colored, powdered and odorless)	0.072
76-82	Benzene: Diethyl ether (5:95)	-	
83-88	Diethyl ether	-	
89-94	Diethyl ether: Ethyl acetate (95:5)	-	
95- 99	Diethyl ether: Ethyl acetate (90:10)	LP5 (white colored powder and odorless)	0.03
100-105	Diethyl ether: Ethyl acetate (80:20)	-	
106-110	Diethyl ether: Ethyl acetate (70:30)	-	

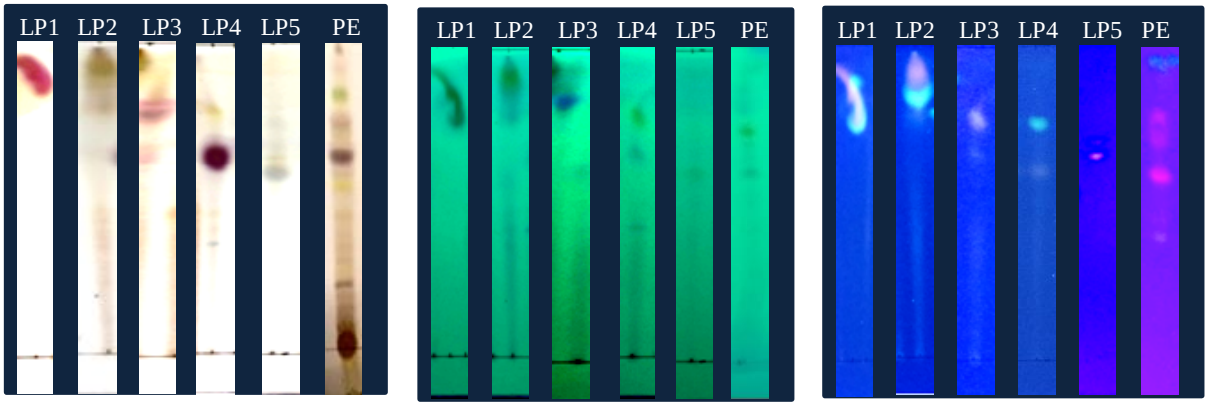
Calculated on weight basis of dried petroleum ether of *Aloe vera* gel used in extraction

3.3.3. Chromatographic analysis

3.3.3.1. Thin layer chromatography (TLC) analysis

Five partially-purified isolates were obtained after pooling the 110 fractions based on their TLC analysis and named as LP1 to LP5. All the partially purified isolates were creamish- white in

colour and they were odourless. Among the various solvent systems tested, best results were obtained in the solvent system Toluene: Ethyl acetate: Methanol: Formic acid (7.75:1.5:0.25:0.5). The spots were visualized in UV chamber (254, 365 nm) and derivatised by anisaldehyde-sulphuric acid spraying reagent. Post derivatization, characteristic colours were developed on the TLC plates: LP1-reddish pink, LP2-brown, LP3-pink, LP4- purple brown and LP5 – grey. Based on the colour, the nature of the compounds was identified to be fatty acid esters, phytosterols and terpenoids. The Rf values of the spots were found to be LP1-0.83, LP2-0.78, LP3-0.72, LP4-0.58 and LP5-0.50 and they were matched with the spots of petroleum ether extract of *Aloe vera* gel (Figure 3.1). The partially purified isolates (LP1, LP2, LP3, LP4 and LP5) were further analysed by GC/MS analysis.



(A) (B) (C)

Figure 3.1: TLC analysis of partially-purified isolates from petroleum ether extract of *Aloe vera* gel (A) under visible light- post derivatization with anisaldehyde-sulphuric acid reagent; (B)- under UV short wave length; (C)- under UV long wave length.

3.3.3.2. Gas Chromatography- Mass Spectrometry (GC/MS) analysis

GC/MS analysis of all the isolates were performed using protocol as mentioned in material and methods section. The detailed list of phytocomponents identified in the petroleum ether extract of *Aloe vera* gel along with the corresponding retention time, name of the compound, CAS number of the compound, molecular formula, molecular weight as well as their relative abundance, which was expressed in terms of peak area% are presented in Table 3.5. Based upon the peak area %, the content of n-Hexadecanoic acid, β-Sitosterol, Campesterol acetate, Stigmasterol acetate and Cholesta-3,5-diene in the petroleum ether extract of *Aloe vera* gel was found to be were 124.8 $\pm$ 5.8, 11.232 $\pm$ 0.92, 15.6 $\pm$ 0.541, 156 $\pm$ 12.08 and 27.456 $\pm$ 0.941 µg/g, respectively.

Table 3.5. Phytochemical constituents identified in the petroleum ether extract of *Aloe vera* gel using gas chromatography-mass spectrometry. CAS: chemical abstract service

Sl. No.	RT (min)	Name of the compound	CAS	Molecular formula	Molecular weight	Peak Area%
1.	7.915	Tetradecanoic acid	544-63-8	$C_{14}H_{28}O_2$	228	5.57
2.	8.927	Pentadecanoic acid	1002-84-2	$C_{15}H_{30}O_2$	242	3.66
3.	12.484	n-Hexadecanoic acid	57-10-3	$C_{16}H_{32}O_2$	256	20.1
4.	13.095	Octadecanoic acid	57-11-4	$C_{18}H_{36}O_2$	284	4.7
5.	13.771	Nonadecanoic acid	646-30-0	$C_{19}H_{38}O_2$	298	1.45
6.	14.273	Oleic acid	112-80-1	$C_{18}H_{34}O_2$	282	2.21
7.	16.537	Eicosanoic acid	506-30-9	$C_{20}H_{40}O_2$	312	3.38
8.	18.207	Cholesta-3,5-diene	747-90-0	$C_{27}H_{44}$	368	1.8
9.	19.260	Stigmastan-3,5-diene	4970-37-0	$C_{29}H_{48}$	396	1.09
10.	19.561	Campest-5-en-3beta-ol	474-62-4	$C_{28}H_{48}O$	400	3.6
11.	19.782	Campesterol acetate	1900-53-4	$C_{30}H_{50}O_2$	442	2.5
12.	21.520	γ- Sitosterol	83-47-6	$C_{29}H_{50}O$	414	17.4
13.	22.171	β- Sitosterol	83-46-5	$C_{29}H_{50}O$	414	25.7
14.	22.839	Cholest-5-en-3-ol (3.beta.)-, propanoate	633-31-8	$C_{30}H_{50}O_2$	442	0.8
15.	26.512	Stigmasterol acetate	4651-48-3	$C_{31}H_{50}O_2$	454	4.4
16.	27.148	Cholest-5-en-3-ol (3.beta.)-, 3,5-dinitrobenzoate	25279-63-4	$C_{34}H_{48}N_2O_6$	580	1.03

17.	28.627	22,23-Dibromostigmasterol acetate	50633-49-3	$C_{31}H_{50}Br_2O_2$	612	0.61

Following this, GC/MS analysis of the partially purified isolates were performed. The detailed list of phytocomponents identified in the LP1, LP2, LP3, LP4 and LP5 along with the corresponding retention time, name of the compound, CAS number of the compound, molecular formula, molecular weight as well as their relative abundance, which was expressed in terms of peak area% are presented in Table 3.6, Table 3.7, Table 3.8, Table 3.9 and Table 3.10 respectively. Data demonstrates that partially purified non-polar phytocomponents (PPNPP) of *Aloe vera* gel- LP1, LP2, LP3, LP4 and LP5 contains n-Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate with an abundance of 97.07%, 96.04%, 94.03%, 92.45% and 87.49% respectively.

Table 3.6. Phytochemical constituents identified in LP1 using gas chromatography-mass spectrometry. CAS: chemical abstract service

Sl. No.	**RT (min)**	**Name of the compound**	**CAS**	**Molecular formula**	**Molecular weight**	**Peak Area%**
1.	11.970	n-Octanoic acid isopropyl ester	5458-59-3	$C_{11}H_{22}O_2$	186	0.43
2.	12.490	n-Hexadecanoic acid	57-10-3	$C_{16}H_{32}O_2$	256	97.07
3.	13.723	n-Capric acid isopropyl ester	2311-59-3	$C_{13}H_{26}O_2$	214	1.14
4.	13.775	Nonadecanoic acid	646-30-0	$C_{19}H_{38}O_2$	298	0.83
5.	13.870	Oleic acid	112-80-1	$C_{18}H_{34}O_2$	282	0.53

Table 3.7. Phytochemical constituents identified in LP2 using gas chromatography-mass spectrometry. CAS: chemical abstract service

Sl. No.	**RT (min)**	**Name of the compound**	**CAS**	**Molecular formula**	**Molecular weight**	**Peak Area%**

1.	7.91	Tetradecanoic acid	544-63-8	$C_{14}H_{28}O_2$	228	2.23
2.	13.772	Nonadecanoic acid	646-30-0	$C_{19}H_{38}O_2$	298	1.73
3.	18.20	Cholesta-3,5-diene	747-90-0	$C_{27}H_{44}$	368	96.04

Table 3.8. Phytochemical constituents identified in LP3 using gas chromatography-mass spectrometry. CAS: chemical abstract service

Sl. No.	**RT (min)**	**Name of the compound**	**CAS**	**Molecular formula**	**Molecular weight**	**Peak Area%**
1.	7.853	Tetradecanoic acid	544-63-8	$C_{14}H_{28}O_2$	228	1.78
2.	9.880	Pentadecanoic acid	1002-84-2	$C_{15}H_{30}O_2$	242	1.46
3.	11.754	cis-Vaccenic acid	506-17-2	$C_{18}H_{34}O_2$	282	1.68
4.	17.720	Tetracosane	646-31-1	$C_{24}H_{50}$	338	1.05
5.	19.795	Campesterol acetate	1900-53-4	$C_{30}H_{50}O_2$	442	94.03

Table 3.9. Phytochemical constituents identified in LP4 using gas chromatography-mass spectrometry. CAS: chemical abstract service

Sl. No.	**RT (min)**	**Name of the compound**	**CAS**	**Molecular formula**	**Molecular weight**	**Peak Area%**
1.	9.213	n-Heptadecanoic acid	506-12-7	$C_{17}H_{34}O_2$	270	2.78
2.	14.073	Oleic acid	112-80-1	$C_{18}H_{34}O_2$	282	1.06
3.	16.137	Eicosanoic acid	506-30-9	$C_{20}H_{40}O_2$	312	0.23

5.	19.561	Campest-5-en-3beta-ol	474-62-4	$C_{28}H_{48}O$	400	1.32
6.	22.171	β- Sitosterol	83-46-5	$C_{29}H_{50}O$	414	92.45
7.	27.595	Cholest-5-en-3-ol (3.beta.)-, tetradecanoate	1989-52-2	$C_{41}H_{72}O_2$	596	2.16

Table 3.10. Phytochemical constituents identified in LP5 using gas chromatography-mass spectrometry. CAS: chemical abstract service

Sl. No.	**RT (min)**	**Name of the compound**	**CAS**	**Molecular formula**	**Molecular weight**	**Peak Area%**
1.	9.532	Tetracosanoic acid	557-59-5	C24H48O2	368	3.07
2.	18.315	Isocholesteryl methyl ether	29944-53-4	C28H48O	400	4.41
3.	21.520	γ- Sitosterol	83-47-6	$C_{29}H_{50}O$	414	5.03
4.	26.512	Stigmasterol acetate	4651-48-3	$C_{31}H_{50}O_2$	454	87.49

The mass spectra and molecular structures of the major phytocomponents present in LP1, LP2, LP3, LP4 and LP5 respectively has been depicted in Figure 3.2. Data reveals that the major phytocomponent of LP1 was n-Hexadecanoic acid. It exhibited molecular formula- $C_{16}H_{32}O_2$, molecular weight- 256, retention time-12.490. GC/MS fragment: The peak at 12.490 minutes had a mass [M+] 256. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments m/z 40, 41, 43, 60, 73, 85, 98, 115, 129, 143, 157, 171, 185, 199, 213, 227, 239 and 256 in EI pattern (Figure 3.2 A).

The major phytocomponent of LP2 was Cholesta-3,5-diene. It exhibited molecular formula- $C_{27}H_{44}$, molecular weight- 368, retention time-18.20. GC/MS fragment: The peak at 18.20 minutes had a mass [M+] 368. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments m/z 27, 41, 43, 57, 81, 95, 107, 120, 147, 159, 173, 199, 213, 228, 247, 255, 274, 297, 314, 353, 368 in EI pattern (Figure 3.2 B).

The major phytocomponent of LP3 was Campesterol acetate. It exhibited molecular formula- $C_{30}H_{50}O_2$, molecular weight- 442, retention time-19.795. GC/MS fragment: The peak at 19.795 minutes had a mass [M+] 442. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments m/z 27, 41, 43, 57, 81, 95, 107, 120, 147, 159, 173, 199, 213, 228, 247, 255, 274, 297, 314, 353, 368 in EI pattern (Figure 3.2 C).

The major phytocomponent of LP4 was b-Sitosterol. It exhibited molecular formula- $C_{29}H_{50}O$, molecular weight- 414, retention time-22.171. GC/MS fragment: The peak at 22.171 minutes had a mass [M+] 414. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments m/z 18, 41, 48, 57, 81, 95, 105, 119, 133, 145, 159, 173, 185, 199, 213, 231, 255, 273, 289, 303, 315, 329, 341, 354, 367, 381, 396 and 414 in EI pattern (Figure 3.2 D).

The major phytocomponent of LP5 was Stigmasterol acetate. It exhibited molecular formula- $C_{31}H_{50}O_2$, molecular weight- 454, retention time-26.512. GC/MS fragment: The peak at 26.512 minutes had a mass [M+] 454. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments m/z 41, 48, 69, 81, 95, 105, 119, 133, 145, 159, 173, 187, 199, 213, 228, 239, 255, 267, 282, 296, 309, 323, 337, 351, 365, 382, 394, 412 and 454 in EI pattern (Figure 3.2 E).

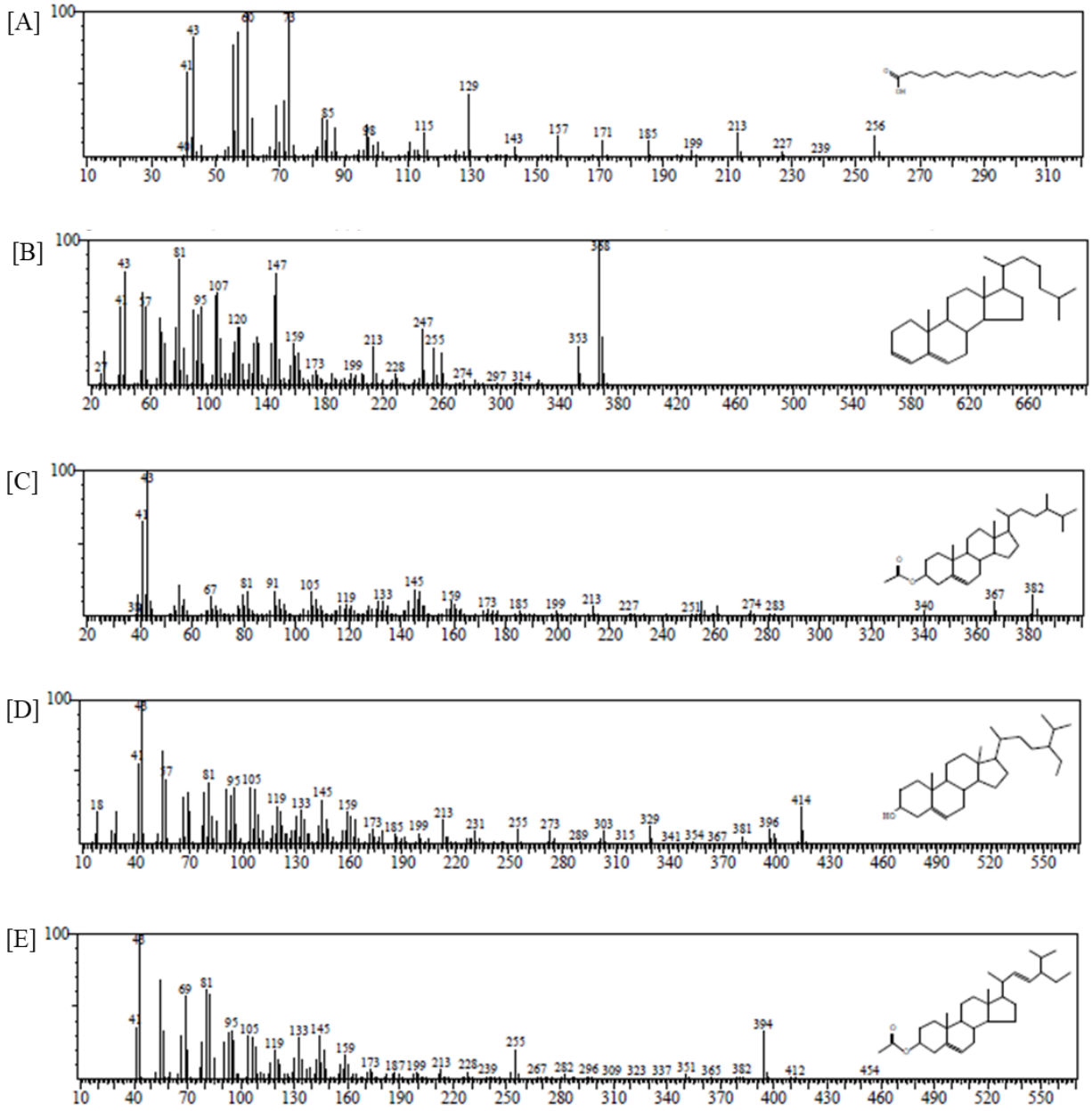


Figure 3.2: Product ion spectrum stating the structure of the major phytocomponents identified in LP1, LP2, LP3, LP4 and LP5 by GC/MS and NIST Library in [A] n-Hexadecanoic acid, [B] Cholesta-3,5-diene, [C] Campesterol acetate, [D] β-Sitosterol and [E] Stigmasterol acetate

3.3.3.3. High Performance liquid chromatography (HPLC) analysis

The purity of the major phytocompounds present in LP1, LP2, LP3, LP4 and LP5 was further, confirmed by Reverse-phase HPLC by matching the peak obtained in the sample with that of the respective standards at the standardized retention time (Figure 3.3). The analytical HPLC profile of LP1, LP2, LP3, LP4 and LP5 measured at 205 nm indicated presence of n-

Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate with high purity as shown by prominent peaks at a retention time of 12.02, 18.9, 20.37, 23.1 and 26.03 minutes respectively.

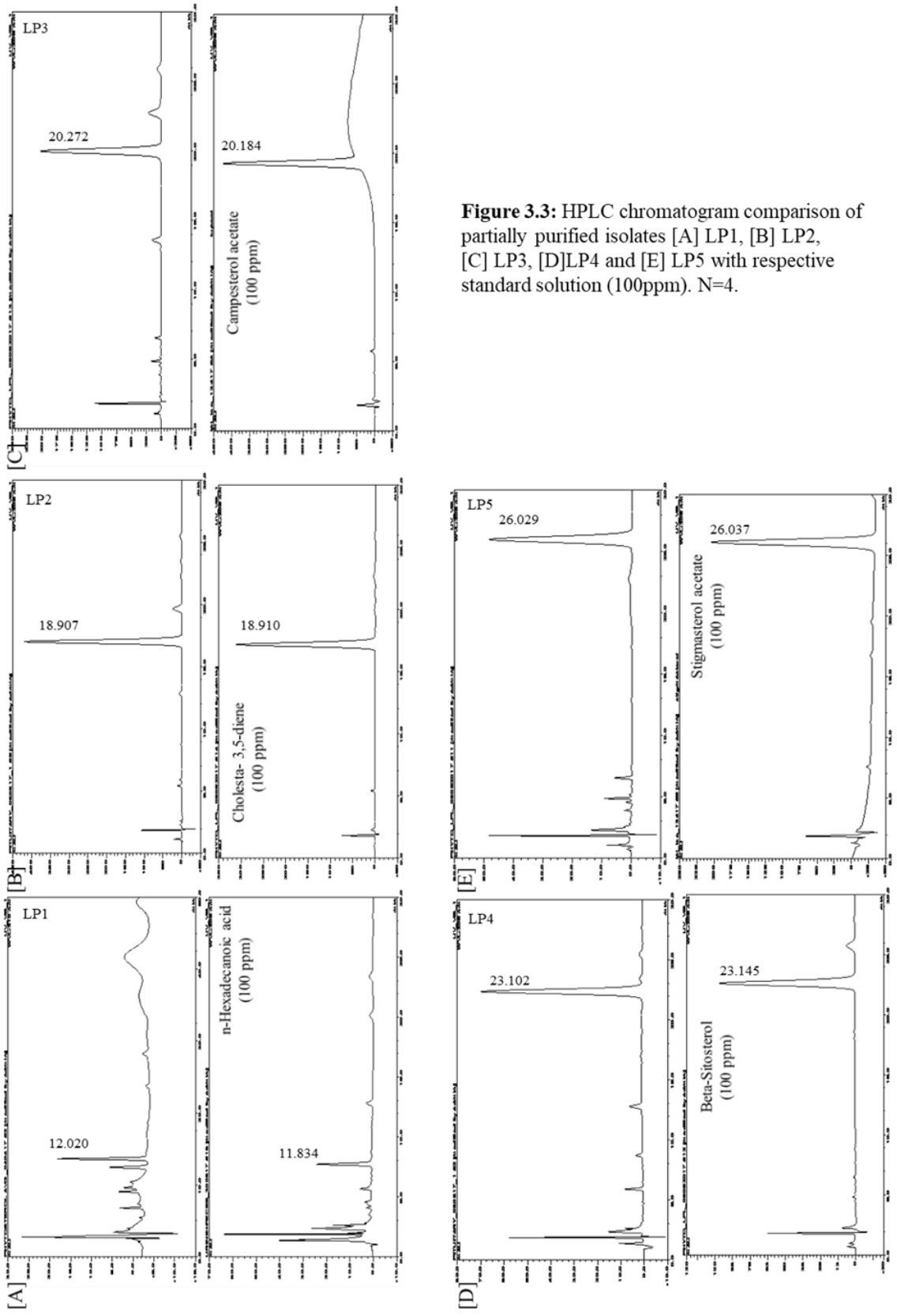


Figure 3.3: HPLC chromatogram comparison of partially purified isolates [A] LP1, [B] LP2, [C] LP3, [D]LP4 and [E] LP5 with respective standard solution (100ppm). N=4.

3.3.3.3.1. Method validation for HPLC analysis

The present investigation was aimed to develop and validate bioanalytical HPLC method for simultaneous estimation of n-Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate (Fig. 3.4) from plant as well as biological matrices. The detailed methodology followed for sample preparations for HPLC analysis has been provided in Chapter 2. For the estimation of analytes present in different samples, a suitable and validated method has to be developed. Selection of appropriate wavelength is necessary to determine the non-polar phytocomponents simultaneously in a single HPLC run. Thus, the working standard solutions of n-Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate, in methanol were scanned over the range of 200-400 nm. At 205 nm, there was no interference from either the mobile phase or baseline disturbance or endogenous substances. Moreover, the response and intensity of n-Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate was found to be better, therefore, 205 nm wavelength was selected for further analytical method development.

The main problem in developing a proper HPLC method for simultaneous estimation of n-Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate in a single run was selection of an appropriate mobile phase, because of differences in their molecular weights and polarity. Therefore, initial trials were performed to optimize the mobile phase composition by taking different ratios of solvents such as ethanol and acetonitrile. This was done to get good separation of analytes and sharp peaks without tailing and interference from the endogenous substances. After several runs, the mobile phase containing ethanol: acetonitrile: 20:80, v/v was selected. The flow rate of 1.5 ml/min and detection wavelength of 205 nm were chosen at which n-Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate gave better response with no interference from endogenous substances and other metabolites (Figure 3.4).

Under these conditions, the retention times of n-Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate were found to be 12.02, 18.9, 20.37, 23.1 and 26.03 minutes respectively with a total run time of 30 min. The representative chromatograms of have been shown in Figure 3.3 and Figure 3.4.

The parameters studied for validation were selectivity, linearity, precision, accuracy, and limit of detection and limit of quantification. The results obtained are as follows.

3.3.3.3.1.1. Linearity and sensitivity

The linear regression equation describing the obtained calibration plots n-Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate showed correlation coefficient greater than 0.98 and linear response over the concentration range of 5-500 ng/mL. Each concentration was repeated thrice on three separate days to obtain the calibration data. Table 3.11 demonstrates the calibration curve equations, correlation coefficient values and LLOQ (lower limit of quantitation) values. LLOQ of the method was found to be 5.0 ng/mL for n-Hexadecenoic acid, Campesterol acetate and Stigmasterol acetate, however, it was found to be 8.0 ng/mL and 6.0 ng/mL for Cholesta-3,5-diene and b-Sitosterol respectively with acceptable accuracy and precision (<15% for each criterion) (Table 3.12). High sensitivity of the developed method was established by determining the concentrations of three spiked calibration standards of the respective standards, with minimum background noise, and better chromatographic resolution, particularly in the LLOQ region. The current method demonstrates high sensitivity for n-Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate over a range of 5-500 ng/mL and therefore, has potential application for numerous pharmacokinetic analyses.

Table 3.11. Calibration curves, correlation coefficients, linear ranges and LLOQ of the non-polar phytocomponents of *Aloe vera* gel

Phytocomponent	Calibration curve	Correlation Coefficient (r^2)	Linear Range (ng/mL)	LLOQ (ng/mL)
n-Hexadecenoic acid	Y= 0.7471X-0.0036	0.9932	5-500	5.0
Cholesta-3,5-diene	Y=0.5707X- 0.0129	0.9918	5-500	8.0
Campesterol acetate	Y= 0.0521X+0.0431	0.9876	5-500	5.0
b-Sitosterol	Y= 1.7824+0.4814	0.9904	5-500	6.0
Stigmasterol acetate	Y= 0.2605X +0.8345	0.9899	5-500	5.0

where, Y is the response (peak area) and X is the concentration

3.3.3.3.1.2. Precision and accuracy

The intra- and inter-day precision of the entire method were assessed by analysing the quality control samples (Table 3.12) during a single day and on three, consecutive days, respectively. The low value of relative standard deviation RSD (%) for all the analytes at all concentrations indicated that the proposed method was precise and accurate within the acceptance limit of 2%.

Phytocomponent	Spiked Concentration (ng/mL)	Intra- Day			Inter-Day		
		Measured (ng/mL)	RE (%)	RSD (%)	Measured (ng/mL)	RE (%)	RSD (%)
n-Hexadecenoic acid	5	5.02 ± 0.06	1.8	3.1	5.01 ± 0.08	2.0	3.9
	100	99.23 ± 6.43	2.0	7.8	102.11 ± 3.24	0.9	8.6
	350	352.6 ± 8.46	-0.7	1.8	351.9 ± 6.21	1.3	1.4
Cholesta-3,5-diene	8	8.01 ± 0.15	6.5	4.1	8.00 ± 0.13	-0.4	5.2
	100	101.34 ± 8.13	-0.1	0.8	102.12 ± 6.27	1.2	0.6
	350	348.21 ±10.67	4.3	4.8	350.02 ± 19.23	-3.7	4.4
Campesterol acetate	5	4.97 ± 0.09	1.0	4.2	5.03 ± 0.02	2.0	3.9
	100	101.11 ± 9.43	-0.8	7.8	102.41± 7.24	0.9	8.6
	350	350.3 ± 6.12	-1.5	3.0	349.2 ± 15.21	1.3	3.4
b-Sitosterol	6	6.03 ± 0.02	4.2	9.4	5.98 ± 0.09	-0.5	2.4
	100	99.82 ± 3.27	0.5	10.9	102.04 ± 7.38	6.5	6.6
	350	351.12 ±18.17	1.3	3.0	350.86 ± 21.52	-2.4	4.9
Stigmasterol acetate	5	5.01 ± 0.02	0.6	2.6	5.01 ± 0.19	2.0	2.7
	100	102.23 ± 3.78	-0.5	5.2	101.33± 5.21	-0.4	4.9
	350	348.6 ± 10.73	-0.7	1.8	348.7 ± 12.82	1.3	1.4

Table 3.12. The intra-day and inter-day accuracies and precisions of the non-polar phytocomponents of *Aloe vera* gel at low, medium, and high concentration levels
The values are represented as Mean ± SEM. N=6. RSD- Relative Standard deviation was used to assess precision and RE- Relative Error was used to assess accuracy.

3.3.3.3.1.3. Recovery

The absolute recovery was calculated by comparing the peak areas of the prepared quality control samples with those of the standard solutions for the low, medium and high-quality control samples. Details of the quality control sample preparation has been provided in Chapter 2. The extraction recoveries (%) and matrix effect (%) of n-Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate at 3 different concentrations has been provided in Table 3.13. The recovery of all the analytes by using the described procedure was consistent and efficient.

Table 3.13. Extraction recoveries and matrix effects of the non-polar phytocomponents of *Aloe vera* gel at low, medium, and high concentration levels

Phytocomponent	**Spiked Concentration (ng/mL)**	**Extraction Recovery (%)**	**RSD (%)**	**Matrix effect (%)**	**RSD (%)**
n-Hexadecenoic acid	5	97.78 ± 1.75	2.6	112.03 ± 3.54	3.0
	100	86.95 ± 3.26	3.2	102.59 ± 3.00	10.2
	350	90.23 ± 3.05	4.8	111.49 ± 6.28	5.6
Cholesta-3,5-diene	8	85.06 ± 2.69	0.8	98.77 ± 5.84	7.2
	100	76.08 ± 9.76	6.7	113.02 ± 10.23	5.1
	350	99.77 ± 7.02	5.6	108.23 ± 9.34	9.8
Campesterol acetate	5	94.72 ± 0.76	4.8	112.31 ± 11.58	11.1
	100	89.78 ± 8.65	9.6	110.84 ± 13.78	5.4
	350	87.96 ± 3.29	11.3	95.69 ± 6.53	6.9
b-Sitosterol	6	84.07 ± 8.65	3.2	115.44 ± 9.45	9.8
	100	91.38 ± 12.2	8.9	113.90 ± 5.95	5.7

	350	95.43 ± 5.94	1.8	114.36 ± 6.34	2.6
Stigmasterol acetate	5	79.30 ± 6.32	8.5	98.55 ± 7.84	4.8
	100	94.32 ±1.8	4.3	104.11 ± 6.91	3.0
	350	88.84 ± 9.11	3.6	89.2 ± 12.58	12.1

The values are represented as Mean + SEM. N=6.

The developed HPLC method was successfully applied to quantitatively monitor the pharmacokinetics of n-Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate after a single oral dose of 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel to adult female Charles Foster rats.

Poor pharmacokinetic characteristics are a major reason why many drugs fail to reach the market (Darvas et al. 2002). As a result, it is now critical to develop lead compounds that are easily absorbed orally, transported to their desired site of action, are not easily metabolised into toxic metabolic products before reaching the targeted site of action, and are easily eliminated from the body before accumulating in sufficient amounts to cause adverse side effects. Hence, Pharmacokinetic study was performed to study how the rat's body reacts to the major non-polar phytochemicals of *Aloe vera* gel (Abdel-Rahman and Kauffman, 2004). Absorption, distribution, metabolism, and excretion (ADME) are the most important PK activities, which are then augmented by toxicity (ADMET). While ADME attempts to enhance a small molecule's pharmacological efficacy, toxicology ensures that it does not cause any harm in the form of side effects (Hodgson 2001). In the current study, we analysed the absorption, distribution and excretion of the major non-polar phytochemicals of *Aloe vera* gel by HPLC. Also, the toxic effects on the key liver and kidney markers were evaluated biochemically.

3.3.4. Pharmacokinetics of Non-polar Phytocomponents of *Aloe vera* gel in rats

The validated HPLC method was successfully used to determine the concentration of n-Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate in rat plasma following a single oral dose of 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel. Figure 3.4 shows the representative HPLC chromatograms. Figure 3.5 shows the plasma concentration of n-Hexadecenoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate at specific time points after administration. The values of all the major pharmacokinetic parameters such as highest plasma

concentration (Cmax), time required for maximum plasma concentration (Tmax), area under curve (AUC0-∞), terminal half-life (t1/2), volume of distribution (Vd) and clearance (CL) have been summarized in Table 3.14.

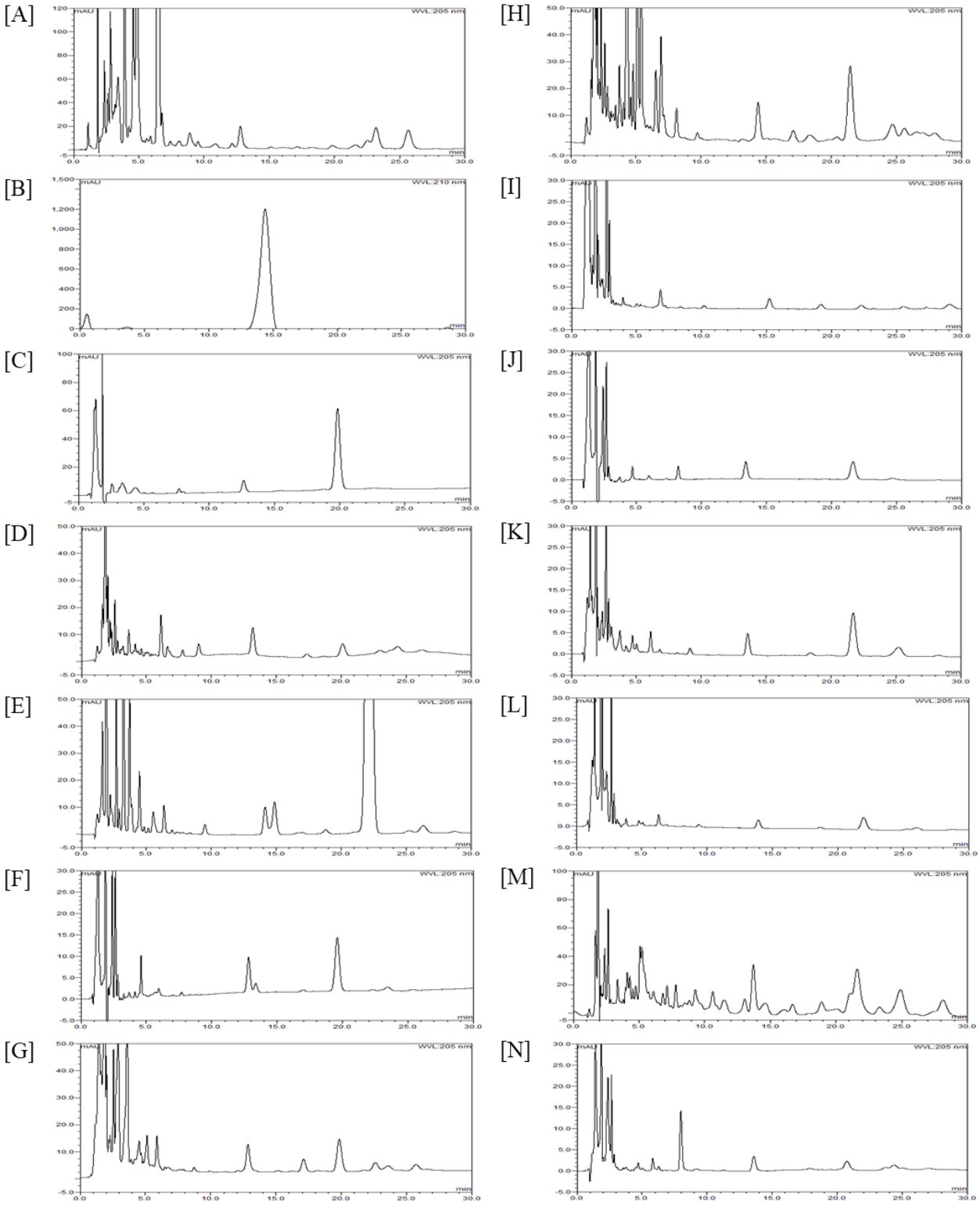


Figure 3.4. Representative HPLC Chromatograms for (A) Petroleum ether extract of *Aloe vera* gel (100 µg/mL); (B) internal standard (5α-cholestane-100ng/mL); (C) blank rat plasma; (D)

rat plasma sample obtained 2.5 hours after a single oral dose of 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel ; (E) hypothalamus, (F) pituitary, (G) adrenal, (H) ovary, (I) uterus, (J) skeletal muscles, (K) liver, (L) kidney samples obtained 5 hours after a single oral dose of 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel, (M) faeces and (N) urine samples after a single oral dose of 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel.

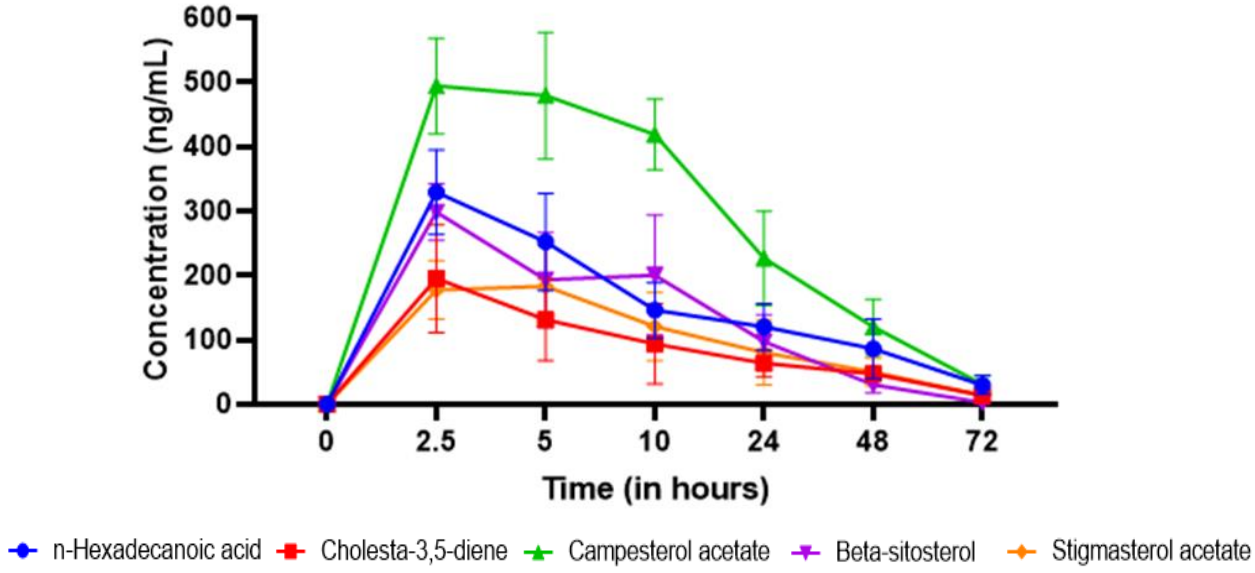


Figure 3.5: Plasma concentration–time curves of non-polar phytocomponents of *Aloe vera* gel in rats after a single oral administration of 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel. The values are represented as Mean ± SEM. N=6.

Table 3.14. Pharmacokinetic parameters of n-Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate in the plasma of rats after a single oral administration of 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel

Parameter	**n-Hexadecanoic acid**	**Cholesta-3,5-diene**	**Campesterol acetate**	**b-Sitosterol**	**Stigmasterol acetate**
Cmax (ng/mL)	376.43 ± 40.78	312 + 69.37	494.18 ± 39.28	272.49 ± 37.88	99.12 ± 58.34
Tmax (hrs)	2.5 ± 0.31	2.5 ± 0.45	2.5 ± 0.38	5 ± 0.41	2.5 ± 0.33
$T_{1/2}$ (hrs)	18.5 ± 0.09	15.66 ± 0.07	13.6 ± 0.05	10.9 ± 0.11	16.64 ± 0.05

AUC (hr. ng/mL)	12171.43 ± 352.74	4686.72 ± 142.96	9155.82 ± 233.35	6775.8 ± 328.81	1609.04 ± 451.27
V_D (L)	2.656 ± 0.61	4.155 ± 0.97	4.703 ± 0.48	3.67 ± 0.58	17.25 ± 0.4
CL (L/hr)	0.082 ± 0.003	0.1507± 0.02	0.109± 0.009	0.183± 0.04	0.621± 0.02

The values are represented as Mean ± SEM. N=6.

After understanding the absorption pattern of the non-polar phytocomponents of *Aloe vera* gel in the rat plasma, the distribution pattern of these phytochemicals in various tissues of the body were undertaken. Tissue distribution studies in animals help to determine the distribution and accumulation of the compound and/or metabolites, especially in relation to potential sites of action (Singh, 2018).

3.3.5. Tissue distribution of Non-polar Phytocomponents of *Aloe vera* gel in rats

Tissue distribution to the hypothalamus, pituitary, ovary, adrenal, uterus, skeletal muscles, liver and kidneys were assessed at three different time points (5, 24 and 48 hrs) after a single oral administration of 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel in female Charles Foster rats and the results are presented schematically in Figure 3.6. Data from the representative HPLC chromatograms provided in Figure 3.4 demonstrates that the non-polar phytocomponents underwent a rapid and wide distribution to all the examined tissues. Levels of Campesterol acetate in steroidogenic tissues like Ovaries and Adrenal were markedly higher than other tissues, whereas, n-Hexadecenoic acid was found to be distributed more in the neuroendocrine organs of the body. i.e., Hypothalamus and Pituitary, suggesting that they can cross the blood-brain barrier and play a role as a neuroendocrine regulator. All the phytocomponents were found to be distributed to Liver, suggesting that they are bio transformed in the liver and processed for metabolism, which is further excreted out through urine or faeces.

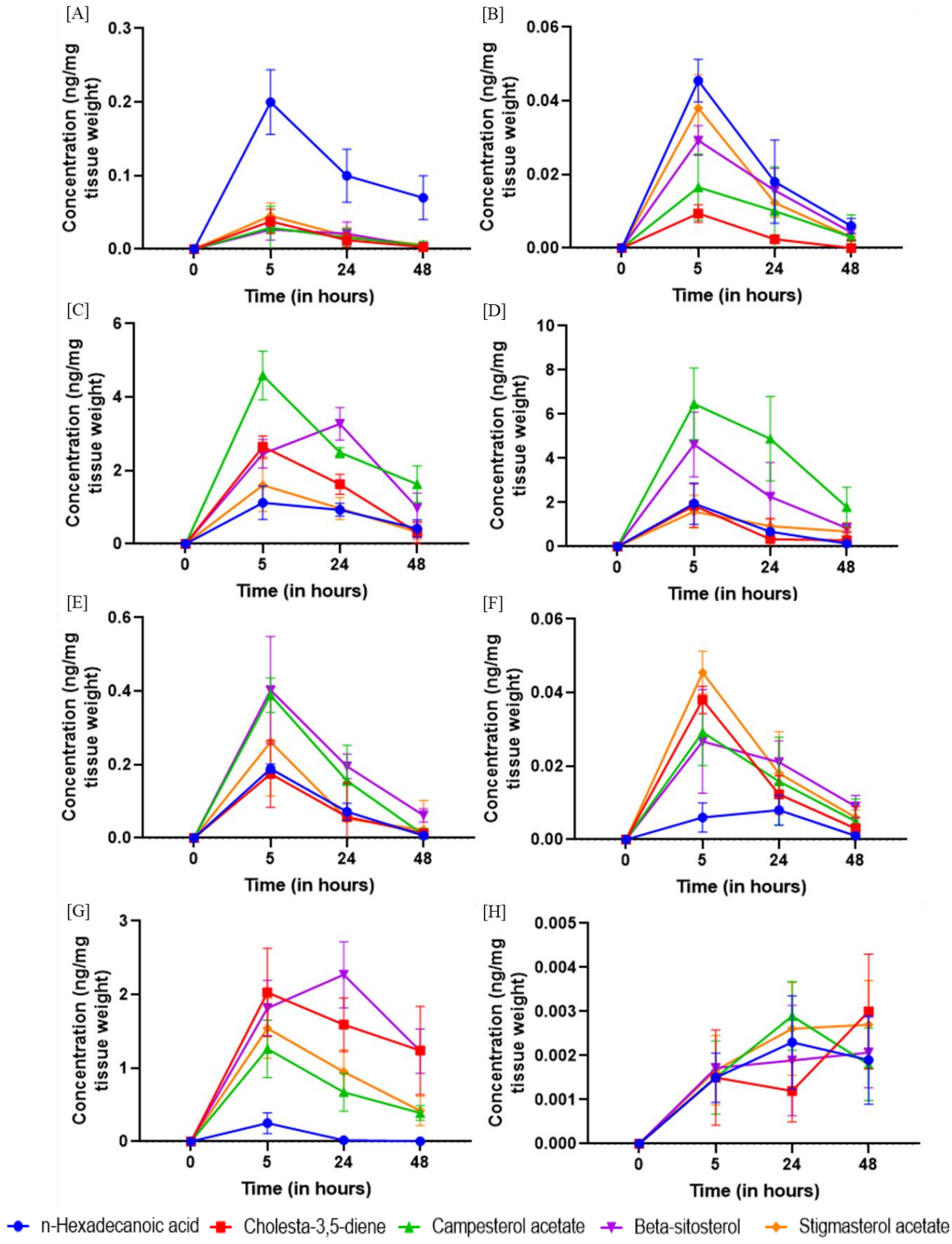


Figure 3.6: Tissue distribution of non-polar phytocomponents of *Aloe vera* gel in [A] Hypothalamus, [B] Pituitary, [C] Adrenal, [D] Ovary, [E] Uterus, [F] Skeletal Muscles, [G]

Liver and [H] Kidney of rats a single oral administration of 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel. The values are represented as Mean ± SEM. N=6.

3.3.6. Excretion of Non-polar Phytocomponents of *Aloe vera* gel in rats

The excretion pattern of the non-polar phytochemicals in the faeces and urine after a single oral administration of 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel has been given in Figure 3.7. The representative chromatogram is provided in Figure 3.4. Excretion data demonstrates that the unmetabolized non-polar phytocomponents are eliminated by the faeces and they are almost cleared out from the body by 72 hours.

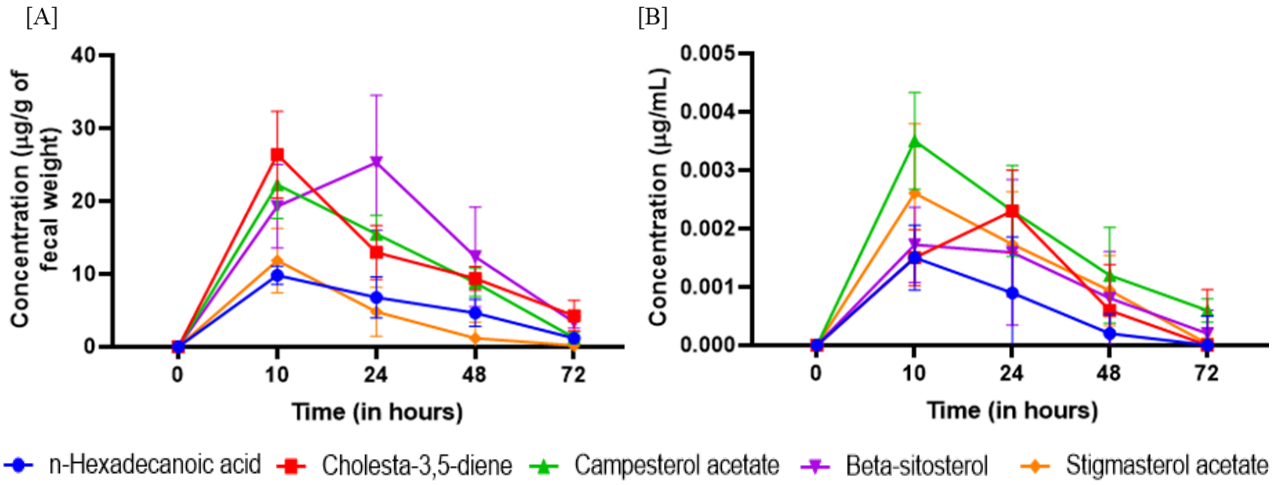


Figure 3.7: [A] Faecal and [B] urinary excretion of unmetabolized non-polar phytocomponents after oral administration of 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel. The values are represented as Mean ± SEM. N=6.

3.3.5. Pharmacodynamic analysis of Non-polar Phytocomponents of *Aloe vera* gel in rats

Pharmacodynamic analysis mainly studies the potentiality of drug to elicit a response. The main aim of this work was to identify the role of non-polar phytocomponents present in *Aloe vera* gel that could effectively modulate the steroidogenic milieu of an individual. In the current study, we evaluated the effect of non-polar phytochemicals on the hormone profile of the rats after oral administration of 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel. Data from Figure 3.8 demonstrates that the non-polar phytocomponents present in petroleum ether extract significantly reduced the testosterone levels and increased the 17 b estradiol and progesterone levels, suggesting that the phytochemicals possess steroid modulatory properties.

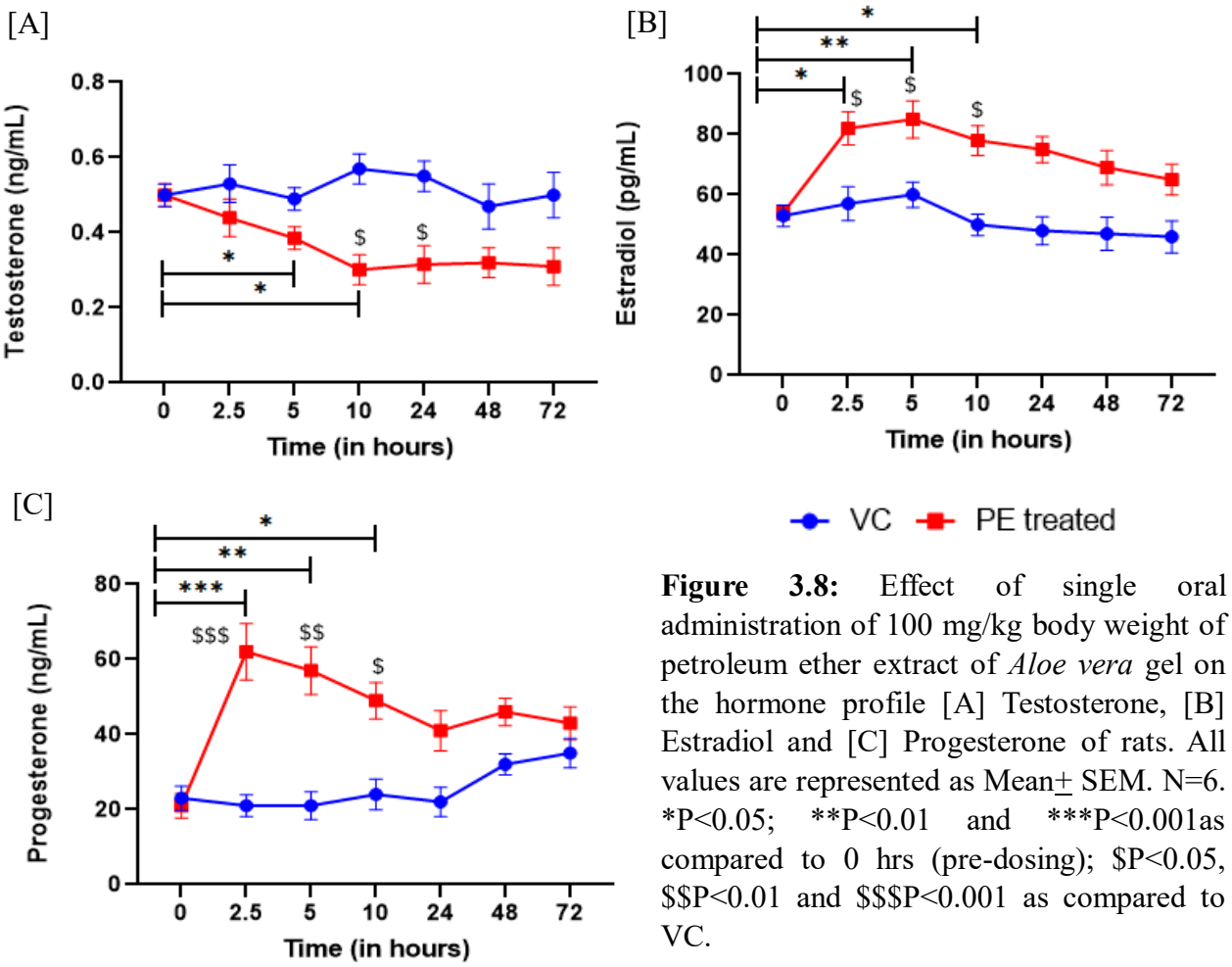


Figure 3.8: Effect of single oral administration of 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel on the hormone profile [A] Testosterone, [B] Estradiol and [C] Progesterone of rats. All values are represented as Mean± SEM. N=6. *P<0.05; **P<0.01 and ***P<0.001as compared to 0 hrs (pre-dosing); $P<0.05, $$P<0.01 and $$$P<0.001 as compared to VC.

3.3.6. Analysis of Toxicity Markers

Effect of non-polar phytocompounds of *Aloe vera* gel on liver and kidney toxicity markers- ALT, AST and creatinine were examined in the plasma of rats. Data from Figure 3.9 demonstrates that there was no significant change in the activities of ALT, AST as well as creatinine levels between the vehicle control group and 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel treated group (Figure 3.9). The observed data suggests that the phytochemicals do not cause any toxic effect on the liver and kidney function (major excretion route).

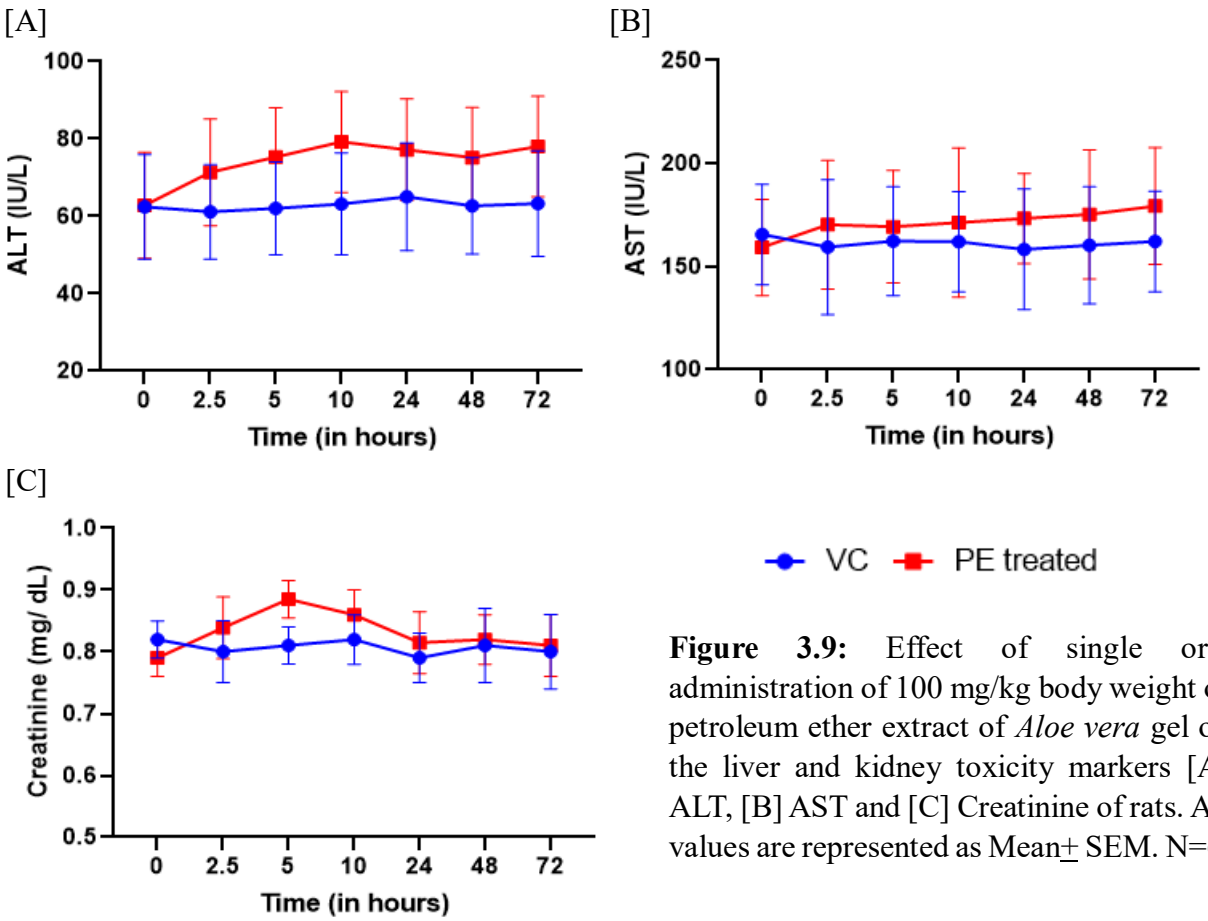


Figure 3.9: Effect of single oral administration of 100 mg/kg body weight of petroleum ether extract of *Aloe vera* gel on the liver and kidney toxicity markers [A] ALT, [B] AST and [C] Creatinine of rats. All values are represented as Mean± SEM. N=6.

3.4. Discussion

The current study lays the foundation for a better understanding of the phytochemical characterization, absorption, tissue distribution, excretion and toxicity of non-polar phytocomponents of *Aloe vera* gel and the evaluation of its clinical application. The study also attempted to isolate few of the major non-polar phytocomponents from the petroleum ether extract of *Aloe vera* gel and characterized them using chromatographic techniques like TLC, GC/MS and HPLC. Literature survey revealed that different phytosterols have been reported to be individually estimated and in combination with other markers from different biological matrices (Lee et al., 2018; Shah et al., 2010; Borkovcová et al., 2009) but there are no reports of simultaneous estimation of n-Hexadecanoic acid, Cholesta-3,5 diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate in plant as well as biological matrices using HPLC. This study for the first time has developed a method to facilitate such analysis which is rapid, sensitive and cost-effective.

Analytical method development is concerned with the development of methods for identifying, separating, and quantifying chemical components. The separation of substances by chromatography is based on variations in compound migration rates between the stationary phase and the mobile phase. The best method should be simple and reproducible, as it may be used by a variety of operators and in a variety of laboratory conditions. Any oral therapeutic agent undergoes first pass metabolism in liver after oral administration (Pond and Tozer,1984). It has been reported that the bioavailability of phytosterols from various dietary sources were low due to the substitutions at C-24 position, leading to inhibition of their absorption from the gastrointestinal system (Delaney et al., 2004). As our method was found to be linear over a plasma concentration range of 5 to 500 ng/mL, it can be used for quantification of the analyte at a lower concentration. Thus, our method could detect non-polar phytocomponents of *Aloe vera* gel systemically even after oral administration, followed by extensive first pass metabolism in the liver, with acceptable accuracy and precision.

To be effective as a drug, the potential molecule must reach its target in the body in sufficient concentration, and stay there in a bioactive form long enough for the expected biologic events to occur (Hodgson 2001). As suggested by FDA, pharmacokinetic and pharmacodynamic assessment is one of the main focuses for optimization in the drug development process (Vaddady et al., 2010). The present study, for the first time, holistically describes the pharmacokinetics, tissue distribution, excretion and toxicity parameters of the major non-polar phytocomponents of *Aloe vera* gel. This study has demonstrated that the non-polar phytocompounds of *Aloe vera* gel are distributed most in the steroidogenic targets like Ovaries and Adrenal. Similar reports from the past have stated that phytosterols are incorporated into the cell membranes of tissues (Awad et al., 2005) and have been found to be highly concentrated in the lungs, adrenal cortex, intestinal epithelia and ovaries (Ogbe et al.,2015).

Data from literature shows that β-Sitosterol has estrogen-like effects and modulates the steroidogenic pathway by altering the P450scc activity, thereby, impairing the conversion of cholesterol to pregnenolone (Gerber et al., 2015). Fish exposed to phytosterols display altered sexual development, changes in hormone production, decreased egg production and decreased spawning rate (Mahmood-Khan and Hall, 2008). Goldfish exposure to 75 µg/L phytosterols resulted in a reduction of reproductive steroid levels and changes in gonadal steroidogenesis (MacLatchy et al., 1997). In sub chronic rodent studies, it has been observed that even at small doses (0.5–50 mg/kg/day) phytosterols caused changes in the weights of reproductive organs. For example, when b-sitosterol was administered subcutaneously, it led to reduced testicular

weight and sperm density in rats (Malini and Vanithakumari, 1991) and increased uterine weights in rats (Malini and Vanithakumari, 1993; Baker et al., 1999) and immature sheep (Ovis aries) (Reed, 2016). Chronic effects of a dietary phytosterols mixture (5mg/kg/ day), containing mainly beta sitosterol, on the reproduction of the mouse demonstrate increased the plasma levels of testosterone and decreased the relative uterine weights in the pups of F (2) and F (4) generations. Furthermore, phytosterol exposure increased the concentrations of plasma estradiol in the female pups of F (3) generation and testicular levels of testosterone in the male pups of F (2) generations (Ryökkynen et al., 2005). Limited evidence from animal studies suggests that very high phytosterol intakes can alter testosterone metabolism by inhibiting 5a-reductase, a membrane-bound enzyme that converts testosterone to dihydrotestosterone, a more potent metabolite (Awad et al., 1998). Dietary intake of Coumesterol decreases the amplitude of LH pulses in ewes (Montgomery et al., 1985). Thereby, suggesting that phytosterols and its metabolites may act as GnRH modulators. In the current study, pharmacodynamic studies have highlighted similar effects of non-polar phytochemicals of *Aloe vera* gel on the hormone profile of rats after a single oral dose of 100mg/kg body weight of petroleum ether extract of *Aloe vera* gel was fed to them by an oral gavage.

3.5. Conclusion

In the present study, freeze dried *Aloe vera* gel was extracted with various non-polar solvents and it was found that petroleum ether extract of *Aloe vera* gel was rich in non-polar phytocomponents like phytosterols, fatty acids, glycosides etc. The extract was further fractionated using column chromatography to derive five partially purified isolates LP1 to LP5. Upon characterization by GC/MS, followed by identification by NIST library, it was found that LP1, LP2, LP3, LP4 and LP5 contains n-Hexadecanoic acid, Cholesta-3,5-diene, Campesterol acetate, b-Sitosterol and Stigmasterol acetate with an abundance of 97.07%, 96.04%, 94.03%, 92.45% and 87.49% respectively. The purity of the isolates was further confirmed by HPLC using respective standards having >99% purity. Also, a validated, rapid and sensitive HPLC method was established, and used to study the pharmacokinetics and tissue distribution of non-polar phytocomponents of *Aloe vera* gel in rats. The aim of the study was to identify phytochemicals from *Aloe vera* gel that have a potential to modulate steroidogenesis, so that they can be used for management of pathologies which involves a dysregulated hormonal milieu. The pharmacokinetic results were corelated with the pharmacodynamic analysis, wherein, Testosterone levels were found to decrease, along with an increase in the Estradiol

and Progesterone levels. The observed effect was due to the absorption of non-polar phytochemicals in the blood as well as tissue distribution to key steroidogenic targets like ovaries and adrenals. More interestingly, treatment with petroleum ether extract of *Aloe vera* gel was found to be safe for consumption. The current study has identified the bioactive non-polar phytochemicals with steroid modulatory properties, however, detailed study involving *"in-silico"*, *"in-vitro"* and *"in-vivo"* models need to be performed to elucidate the molecular targets of these phytochemicals and the mechanism of their bioactivity.