
CHAPTER 6

PHYTOCHEMICAL ANALYSIS OF *ALOE VERA* GEL

6.1 INTRODUCTION

Scientifically, *Aloe vera* is known as *Aloe barbadensis* miller. It belongs to the family of Liliaceae, and is a shrub, perennial, xerophytic, succulent, pea-green color plant. It grows around the world but mainly in dry region. *Aloe vera* has been used for various medicinal purposes in different parts of the world since a long time: Greece, Egypt, India, Mexico, Japan and China (Marshall, 1990). *Aloe vera* contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids (Maharjan and Nampoothiri 2015).

Genus: *Aloe* L.

Species: *Aloe barbadensis* Mill.

Family: Liliaceae

Synonym: *Aloe vera* L.

Flowering season: Before Spring
(February-April)

No. of species: About 500

Native: North Africa

Distribution: Tropical regions



The species of *Aloe* selected for commercial exploitation or selected by the traditional healer, would be based on its local availability and distribution. In South Africa, the most widely distributed *Aloe* species are *Aloe greatheadii* var. *davyana* (Asphodelaceae) and *Aloe ferox* Mill. (Asphodelaceae). Different *Aloe* species have varying phytochemical contents due to species variation, varying climate and soil conditions, hence direct correlation of biological activity would be inaccurate without detailed evaluation of its phytochemicals.

Aloe species have been used for their anti-tumor, anti-infection, anti-inflammatory, anti-oxidant, and laxative effects around the world (Tamura et al. 2009; Prabjone et al. 2006). Several clinical and experimental studies have demonstrated the hypoglycemic effects of *Aloe vera* (Rajasekaran et al. 2004; Yongchaiyudha et al. 1996).

Additionally, there are some reports that *Aloe vera* derived extracts showed a preventive effect against insulin resistance (Pérez et al. 2007) and a lipid-lowering effect (Rajasekaran et al. 2006). However, sufficient documentation regarding phytochemical analysis of *Aloe* has not been reported in context to various efficacies. In this view, development of various novel analytical techniques and validation of the method for the analysis of phytochemical constituents has become an important step of herbal drug standardization.

Data from earlier chapters have suggested that aqueous extract of *Aloe vera* gel helped to restore the fertility index and reduced post implantation loss in PCOS rat model. Thereby, it is important to identify phytocomponents of *Aloe vera* gel responsible for modulating the PCO phenotype. Since few years, intensive studies on isolates and other active phytochemicals extracted from plant species has been used in folkloric medicine for improvement of fertility (Bankole et al. 2008; Nascimento et al. 2000; Shittu 2010). Hence, current research is in search for an ideal folkloric phytochemical medicinal agent with a broad spectrum and proven potential of treating infertility conditions including other causes of infertility with minimal or no side effects as compared to their synthetic counterparts (Bankole et al. 2008; Ashiru 2006; Shittu et al. 2009).

In literature, many studies have suggested that phytosterols may have effects on the reproductive system and they possess estrogenic activity (Rosenblum et al. 1993); (Mellanen et al. 1996). Also, phytosterols have been reported to modulate ovarian steroidogenesis which then altered the ovarian structure-function (Sharpe et al. 2007). Thereby, aim of current chapter was to isolate phytochemical contents of *Aloe vera* gel using systematic methodologies. Use of some validated analytical methods such as HPTLC, HPLC, GC-MS/MS, and LC-MS for the phytochemical characterization of *A. vera* herbal extract has also been highlighted in this chapter along with preliminary actions of phytochemicals on ovarian organ.

6.2 MATERIALS AND METHODS

6.2.1 Collection and authentication of plant sample

During collection, healthy samples were selected, since microbial and other infections

may change the metabolites produced by the plant. Various collection conditions such as developmental stage of the plant sample, month or time or place of collection have been documented in Literature. Herbaria for each plant sample were prepared. Plant samples were authenticated from BARO, Department of Botany, The M.S. University of Baroda and the voucher specimens were deposited. The herbaria and authentication certificate for the plant samples are shown in Figure 6.2. The Authentication numbers for the plant samples were RM-1 and RM-2.

All the materials and methods used in this chapter includes qualitative and quantitative analysis, Thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High pressure liquid chromatography (HPLC), Gas chromatography mass spectrometry (GC MS). Detailed methodology has been discussed in material and methods chapter.

Figure 6.2.1 Photographic records of *Aloe barbadensis* Mill. and authentication certificate from BARO Herbaria of the Department of Botany, The M.S. University of Baroda, Vadodara, Gujarat, India.

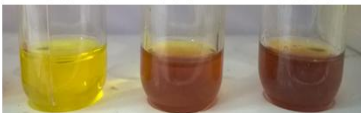

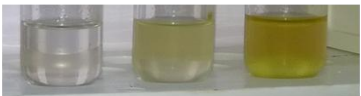






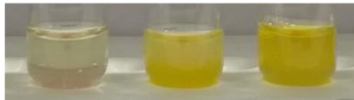
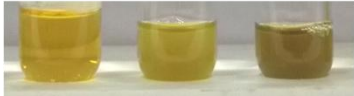
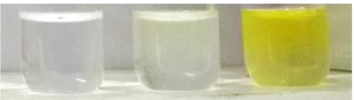
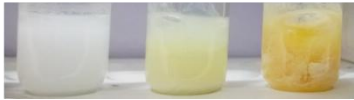

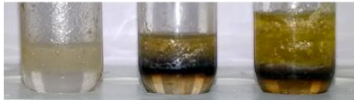

6.3 Results

6.3.1 Preliminary phytochemical analysis of *Aloe vera* gel

In preliminary phytochemical study of *Aloe vera* gel, different qualitative tests were performed for some major groups of phytochemical constituents. In this analysis, *Aloe vera* gel showed the presence of various groups of phytochemical constituents like glycosides, flavonoids, phenolic compounds, alkaloids and phytosterols. The results are shown in table (6.3.1).

Table 6.3.1 Qualitative analysis of *Aloe vera* gel:

Type of phytoconstituents	Test	Representative pictures	Fresh <i>Aloe vera</i> gel (FA)	Formulated <i>Aloe vera</i> gel (FOA)
Steroidal Glycosides	Baliget Test		+	++
	Killer-Killani Test		+	+
Phytosterols	Liebermann-bachmann		+	+
	Salkouski Test		+	+
Terpenoids			+	++

Alkaloids	Mayor's Test	BLANK FA FOA 	-	-
	Wagner's Test	BLANK FA FOA 	-	-
	Dragendroffes test	BLANK FA FOA 	+	+
Phenolic compounds	FeCl ₃ Test	BLANK FA FOA 	+	++
	Gelatin Test	BLANK FA FOA 	+	+
Flavonoids	Lead acetate Test	BLANK FA FOA 	+	++
Saponins	Foam test	BLANK FA FOA 	+	-
Carbohydrates	Molish Test	BLANK FA FOA 	+	+
Proteins	Ninhydrin Test	BLANK FA FOA 	-	-

After qualitative analysis of *Aloe vera* gel, the quantitative analysis of major groups of phytochemical constituents was determined using spectrophotometric method. The calibration curve for determination of the total sterol, flavonoids and total phenolics content in *Aloe vera* gel is shown in Figure 6.3.2 (a), (b), (c) respectively. The results are summarized in table 6.3.2.

Figure 6.3.1(a) Estimation of total sterols
(Sabir et al., 2003)

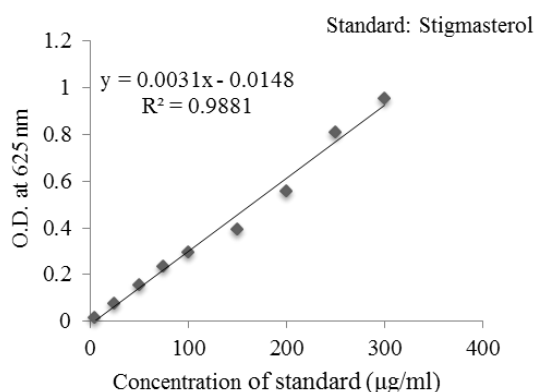


Figure 6.3.1(b) Estimation of Total Flavonoids
(Slinkard and Singleton, 2001)

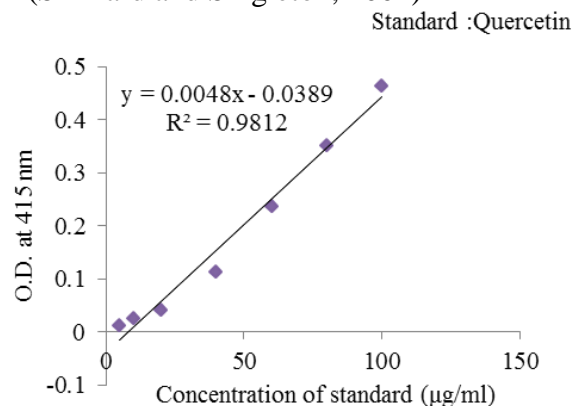


Figure 6.3.1(c) Estimation of Total Phenolics
(Zheng and Wang, 2001)

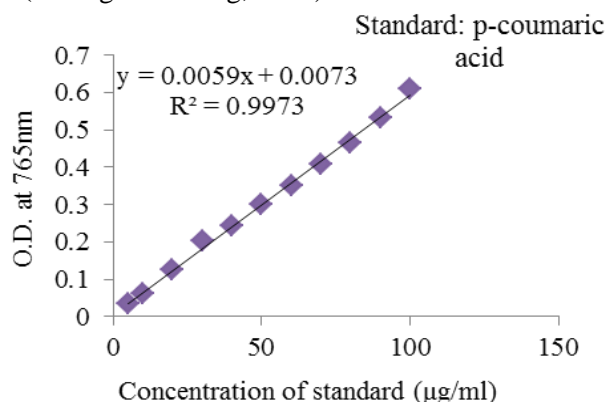


Table 6. 3.2 Concentration of phytocomponents in Fresh and Formulated *Aloe vera* gel

	Fresh <i>Aloe vera</i> gel (µg/ml)	Formulated <i>Aloe vera</i> gel ((µg/ml)
Phytosterols	43.40± 1.762	40.97±7.248
Flavonoids	55.82±10.81	149.4±12.96 **
Polyphenols	31.50±1.166	101.8±5.748***

Formulated *Aloe vera* gel contains turmeric, kadaya gum and lemon as preservatives: Concentration of Flavonoids in turmeric and lemon are 151µg/ml and 2.5µg/ml respectively.

Concentration of Polyphenols in turmeric, kadaya gum and lemon are 113 µg/ml, 24µg/ml and 13µg/ml respectively.

6.3.2 High Pressure Thin Layer Chromatography-Method Development and Optimization

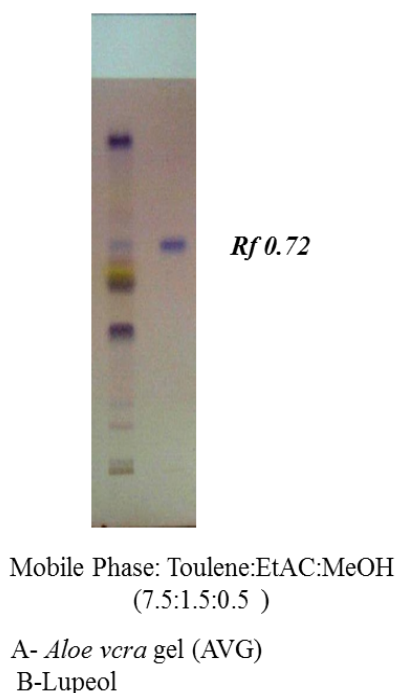
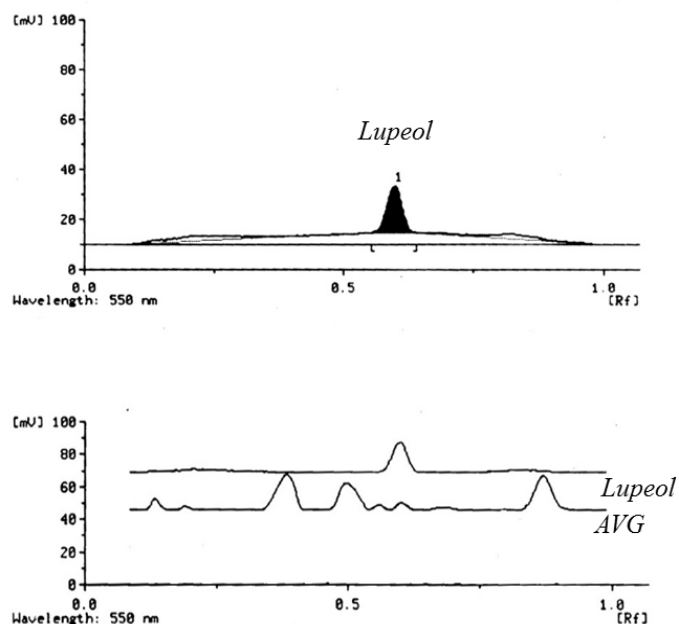
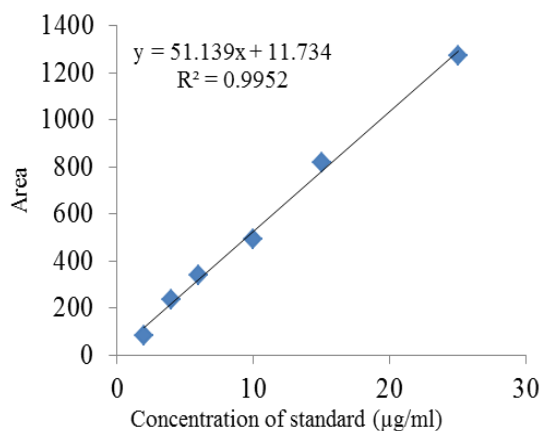
As AVG showed presence of sterols, flavonoids, phenolics in preliminary analysis. Confirmation analysis using HPTLC was preferred. In order to fulfill the objective, different system parameters such as composition of mobile phase, method of sample preparation, detection wavelength were modified to obtain well resolved densitogram. Various solvent systems in different proportions were tried. It was found that the use of Toulene: Ethyl acetate: Methanol (7.5:1.5:0.5) as Mobile Phase demonstrated a compact and sharp band of lupeol with R_f value of 0.72 (Figure 6.3.2).

6.3.2.1 HPTLC fingerprint analysis of Lupeol

The overlay of the HPTLC chromatograms of standard Lupeol and fresh *Aloe vera* gel is shown in figure 6.3.3. This clearly indicates the presence of lupeol in fresh AVG at value R_f 0.72.

6.3.2.2 HPTLC Method Validation

It is very important to develop novel analytical techniques and validate the method for the analysis of active phytoconstituents in plant extract as it will be useful in herbal drug standardization. Hence, we developed HPTLC method for the estimation of lupeol in *Aloe vera* gel and further validated the parameters like linearity, sensitivity, precision, accuracy and robustness.

Figure 6.3.2 Fingerprint of Lupeol and *Aloe vera* gel**Figure 6.3.3 HPTLC densitogram of Lupeol as an active chemical marker****Figure 6.3.4 Linearity of the method for estimation of lupeol****Table 6.3.3: Calibration curves, Limit of detection and Limit of quantitation of Lupeol**

Linear Range [µg/mL]	Coefficient of determination (r^2)	LOD* [µg/mL]	LOQ* [µg/mL]
2-30	0.9952	2	3

6.3.2.3 Linearity plots and detection limits

The linearity for Lupeol was established by plotting the peak area versus six different concentrations (Figure 6.3.5). The data obtained was subjected to regression analysis and the coefficient of determination value of 0.9952 along with the regression equation of $y = 51.13 + 11.73$ was obtained. The LOD and LOQ values for Lupeol were found to be 2 µg/mL and 3 µg/mL, respectively as shown in (Table 6.3.3).

6.3.2.4 Precision, accuracy and robustness

As shown in Table 6.3.4, % RSD value for inter-day and intra-day precision based on peak measurement was found to less than 2 (n=3), hence the methods were found to be precise. The average recoveries of the Lupeol were in the range of 98.93%. Satisfactory recoveries with small % relative standard deviations were obtained, which indicate the accuracy of the method. There was no significant difference in peak areas, retention time and R_f values after small, deliberate variation of the analytical conditions, which evaluated the robustness of the proposed method.

Table 6.3.4: Precision and Accuracy of the method for estimation of lupeol

Sample	Precision (%RSD)		Mean recovery (%) \pm SD	Conc. in (μ g/mL)
	Inter-day	Intraday		
<i>Aloe vera</i> gel	1.98	1.65	98.93 \pm 0.578	0.359

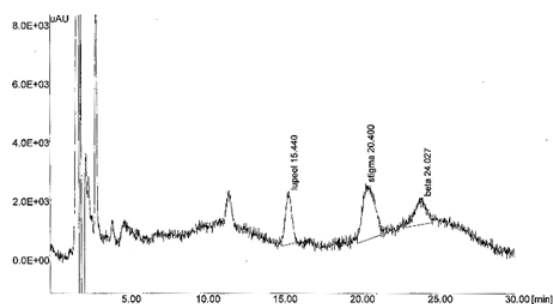
6.3.3. High Pressure Liquid Chromatography (HPLC) analysis of *Aloe vera* gel

Initial experiments were carried out with whole *Aloe vera* gel wherein, several qualitative and quantitative analysis of different phytocomponents was performed. Initial experiments were carried out with whole *Aloe vera* gel wherein, several qualitative and quantitative analysis of different phytocomponents was performed. As most of the reports indicated that methanolic extract is better analyte for analysis, thereby HPLC was performed in methanol extract of AVG using β sitosterol, stigmasterol, Lupeol as standards. (Figure 6.3.5).

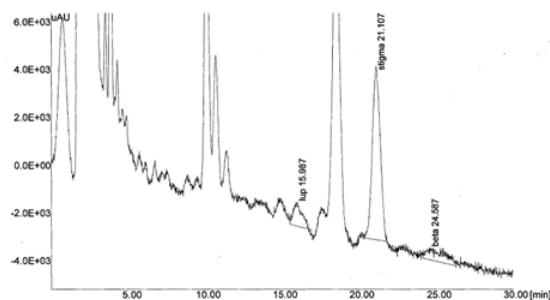
Various *Aloe* spp. have been studied elaborately for its numerous biological properties and commercial use (Maharjan and Nampoothiri, 2015). There are several forms of *Aloe vera* gel like lyophilized powder, pure gel, formulations etc available commercially. *Aloe vera* gel is very volatile in nature and secondary metabolites content present in the gel change frequently upon the processing and storage. Hence, qualitative and quantitative analysis of phytosterols was assessed in two forms of *Aloe vera* gel: *Aloe* formulation and fresh *Aloe* gel as given below in Figure 6.3.5 and Table 6.3.5.

Figure 6.3.5 HPLC chromatograms of *Aloe vera* gel along with the standards

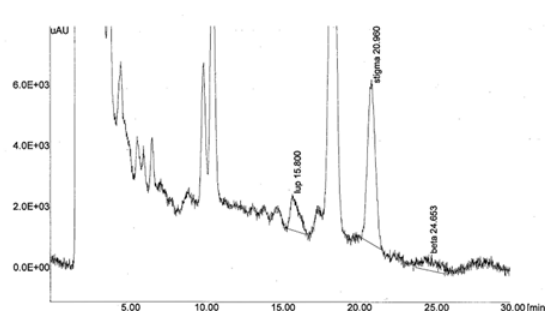
(β -Sitosterol stigmasterol Lupeol)
Standard mixture



Formulated *Aloe*



Fresh *Aloe*

**Table 6.3.5 Quantitative analysis of phytosterol content in *Aloe vera* gel by High Pressure Liquid Chromatography**

Samples	Lupeol ($\mu\text{g/ml}$)	Stigmasterol ($\mu\text{g/ml}$)	β -Sitosterol ($\mu\text{g/ml}$)
Formulated Aloe	0.567	0.605	0.562
Fresh Aloe gel	0.192	0.406	0.140

Quantitative analysis of phytosterols in different forms of *Aloe vera* gel showed that both Fresh *Aloe* and Formulated *Aloe* exhibited good amount of phytosterols in it. Also, it is evident from the previous chapters that Fresh *Aloe vera* gel (AVG) treatment exhibited maximum efficacy both at systemic as well as reproductive organ level in non-pregnant as well as pregnant stage in Letrozole induced PCOS rats. Hence, detailed phytochemical characterization of Fresh *Aloe vera* gel was performed.

Aloe vera is rich in various phytochemical constituents which have diverse biological properties (Hamman, 2008). The phytochemical constituents present in the gel

makes it nutrient rich for various medicinal benefits (Maharjan and Nampoothiri, 2015). The findings of the qualitative and quantitative analysis of *Aloe vera* gel suggested that it contain different phytochemical constituents like polyphenols, sterols, anthraquinones, flavonoids, glycosides etc. Thereby, polarity gradient method based fractionation was performed to obtain the partially purified isolated constituents from the gel with various solvents like petroleum ether, chloroform, ethyl acetate and butanol.

The qualitative and quantitative analysis of each isolated fraction extracted with the organic solvent exhibited that petroleum ether fraction was rich in phytosterol (P₁), Chloroform fraction contained anthraquinones (P₂), Ethyl acetate fraction included flavonoids (P₃) and Butanol extracted fractions had polyphenols (P₄) respectively. (Table 6.3.6, Table 6.3.7).

Table 6.3.6 Qualitative Tests for partially purified components

TEST	P1	P2	P3	P4
Salkouski	+++	+	–	–
Libermann-Burchard	+++	+	–	–
FeCl ₃ Test	–	+++	+	++
Lead Acetate	–	–	+	+++
Alkaline Test	–	+	+++	+

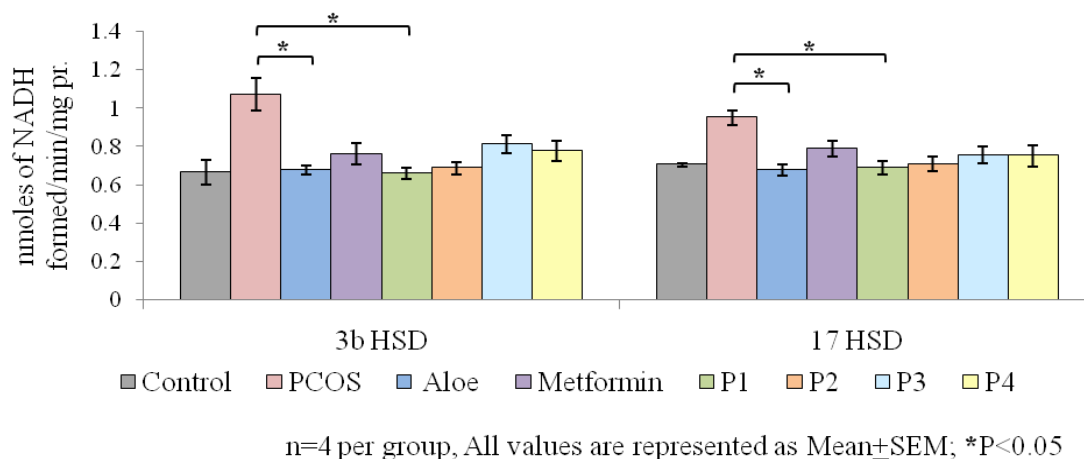
Table 6.3.7 Quantitative analysis of partially purified isolated fractions

Partially purified isolated fractions	Method	Conc. in mg/ml of gel
P1	Sterol (Sabir et al.,2003)	0.76 ± 12.4
P3	Flavonoids (Slinkard & Singleton, 2001)	0.197 ± 6.8
P4	Polyphenol (Wei Zheng, 2001)	0.33 ±8.6

Partially purified isolated fractions -P1, P2, P3 and P4 were found to be rich in sterols, anthraquinones, flavonoids and polyphenols respectively. These phytochemical constituents have been reported to possess different biological properties like anti-

hyperglycemic, anti-oxidant, anti-hyperlipidemic, anti-inflammatory etc. (Hamman 2008) (Kim et al. 2009). Various medicinal plants have been reported to possess the therapeutic potential to manage PCO phenotype. Hence, an *ex-vivo* experiment was performed to check the direct effect of the partially purified isolated extracts containing phytochemical constituents on PCO ovary wherein, ovarian homogenates of PCO ovary were incubated with the partially purified extracts for 1 hour and the enzyme activities of key ovarian steroidogenic enzymes (3β HSD and 17β HSD) was evaluated.

Figure 6.3.6 “Ex vivo” effect of *Aloe vera* gel on steroid enzyme activity in letrozole induced PCOS Rat model



This experiment exhibited that all the fractions had a direct effect on ovarian steroid production. However, Petroleum ether extracted (P₁) fraction demonstrated a significant change (*p<0.05) in PCO ovarian homogenate by modulating the steroidogenic enzyme activities which was comparable to control group (Figure 6.3.6).

Thereby, our study was focused only on the fraction obtained from petroleum ether (P₁) for further experiments to identify different phytocomponents present in P₁ fraction which might be playing a key role in the modulation of ovarian steroidogenesis in PCO condition.

Further, several chromatographic methods like Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC), High Pressure Liquid Chromatography (HPLC) was used to quantitate the content of Phytosterols present in petroleum fraction of *Aloe vera* gel.

For, appropriate separation and resolution of sterols from the other complex phytochemical matrix on TLC plate, advanced technique of TLC namely High Performance Thin layer chromatography was opted.

Figure 6.3.7 Thin Layer Chromatography analysis of P1 fraction of *Aloe vera* gel



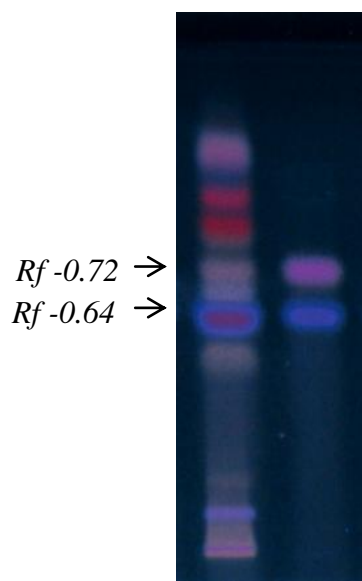
1 – Stigmasterol

2 – P1 fraction

MOBILE PHASE: Toulene:EtAC:MeOH (8.0:1.5:0.75)

For the identification of sterols, P1 fraction was run on preparative TLC plate with respective standard, stigmasterol. After run, TLC plate was derivatized with 10% Methanolic H_2SO_4 reagent. The pink coloured band corresponds to the standard stigmasterol, as observed under visible light.

Figure 6.3.8 HPTLC analysis of P₁ fraction of *Aloe vera* gel



Mobile Phase: Toulene:EtAC:MeOH
(7.5:1.5:0.5)

Track 1- P1 fraction

Track 2 – Mixture of Sterol standard

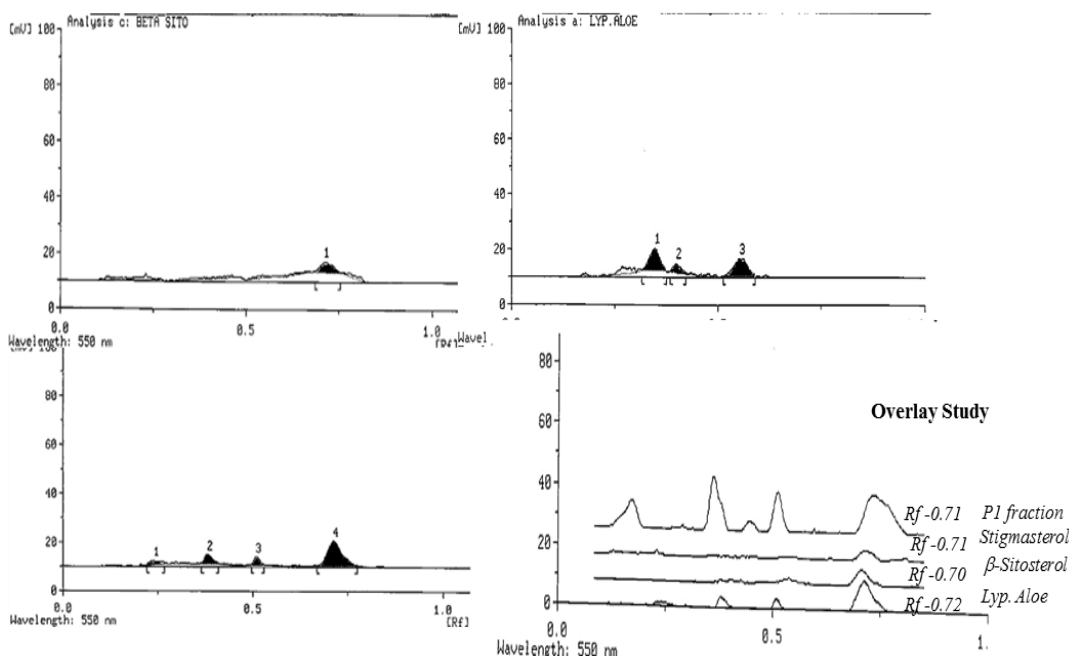
- β -sitosterol (50 ppm)

- Lupeol (50 ppm)

In the HPTLC analysis, 100 ml of P1 fraction was evaporated and again resuspended in 1 ml of petroleum ether solvent. The sample (10 μ L) was spotted on TLC plate and allowed to run in the mobile phase. After development, the plate was

derivatized with 10% methanolic H₂SO₄ reagent, scanned and area under curve (AUC) was determined for the phytosterols. The TLC plate was kept in UV light at 366 nm wherein P1 fraction exhibited well separated phytosterols which were matched with β -sitosterol and lupeol as standards. The plate photo of the same is shown in figure 6.3.8. The R_f values of phytosterols in the sample were calculated and matched with that of the standards (Figure 6.3.9).

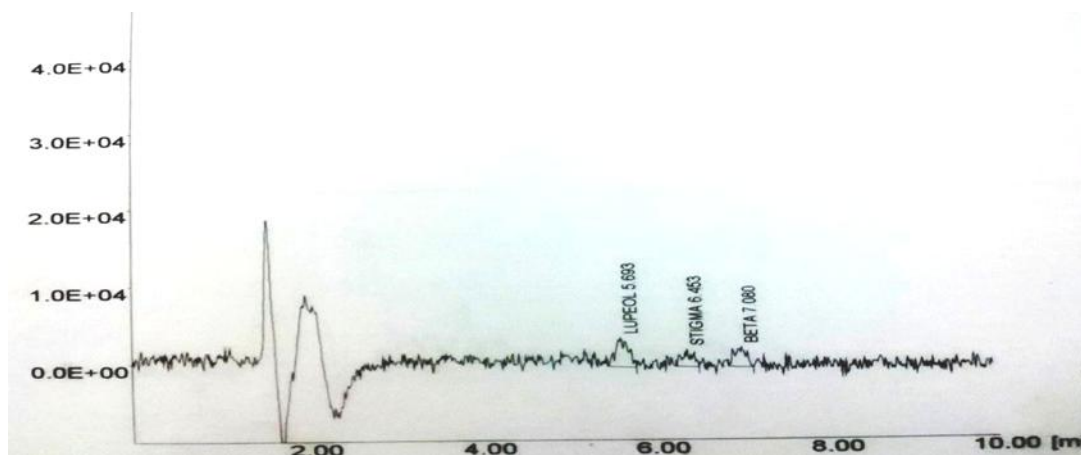
Figure 6.3.9 HPTLC chromatogram of P1 Fraction of *Aloe vera* gel



6.3.3.1 HPLC analysis of P1 fraction of *Aloe vera* gel

It was found that the HPLC is more sensitive chromatographic technique than the HPTLC for the phytochemical analysis. Hence, to estimate phytosterol content in P1 fraction, HPTLC was used, wherein 3 sterol standards: β sitosterol, stigmasterol and Lupeol were injected into the HPLC system. After 30 minutes of run time, the peaks of β sitosterol, stigmasterol and Lupeol were identified with their respective Retention times (RT). Then P1 fraction of *Aloe vera* gel was injected into the system with the help of syringe wherein peaks corresponding to β sitosterol, stigmasterol and Lupeol were obtained within the 30 minutes of run time (Figure 6.3.10).

Figure 6.3.10 HPLC chromatogram of P₁ fraction of *Aloe vera* gel showing the presence of β sitosterol, stigmasterol and Lupeol



After the analysis, the spectral confirmation of the representative peak of phytosterols in the sample was carried out with that of the standard. The RT values were also compared and an AUC value for the representative peak of phytosterol in the sample was obtained. P₁ fraction exhibited presence of all 3 main sterols wherein majorly present sterol was β - sitosterol in the analysis. Lupeol was present in very less concentration in the P₁ fraction (Table 6.3.8).

Table 6.3.8 Quantification of the phytosterols in P₁ fraction of the *Aloe vera* gel using HPLC technique

	Lupeol ($\mu\text{g/ml}$)	Stigmasterol ($\mu\text{g/ml}$)	β -Sitosterol ($\mu\text{g/ml}$)
P ₁ Fraction	0.027	0.410	0.511

In literature, phytosterols are known to have estrogenic property which has ability to bind with estrogen receptor on membrane and modulate the steroid biosynthesis and metabolism (Sharpe et al., 2007). Hence, the phytosterols detected in the P₁ fraction of *Aloe vera* gel could play important role in the steroid modulation in PCOS condition.

6.3.4. Characterization of P1 Fraction of *Aloe vera* gel using GC-MS/MS technique:

In order to confirm the presence of β -Sitosterol and identify some other phytochemical constituents of P1 fraction of the *Aloe vera* gel which might play role in steroid modulation, we also performed Gas chromatography Mass spectroscopy (GC-MS/MS) experiment.

Under standard optimized condition, peak of β -Sitosterol was detected at 35.16 min in GC chromatogram (Figure 6.3.11 and Figure 6.). A mass spectrum of the same is depicted in figure 3.18. On the basis of RT value and fragmentation pattern of some high intensity peaks in the mass spectra, the presence of β sitosterol ($m/z = 414$) in the P1 fraction was confirmed.

Fragmentation pattern (mass spectra) of some other phytochemical constituents separated as peak during GC-MS/MS analysis of the P1 fraction of *Aloe vera* gel were matched with the data from the National Institute of Standards and Technology (NIST) library. The detailed information on the other probable phytochemical constituents detected in the P1 fraction of *Aloe vera* gel (name of the compound, molecular formula and molecular weight) is summarized in the Table 6.3.9.

Figure 6.3.11 GC chromatogram of the P1 fraction of *Aloe vera* gel showing the peak corresponding to β Sitosterol at Rt 35.16 min

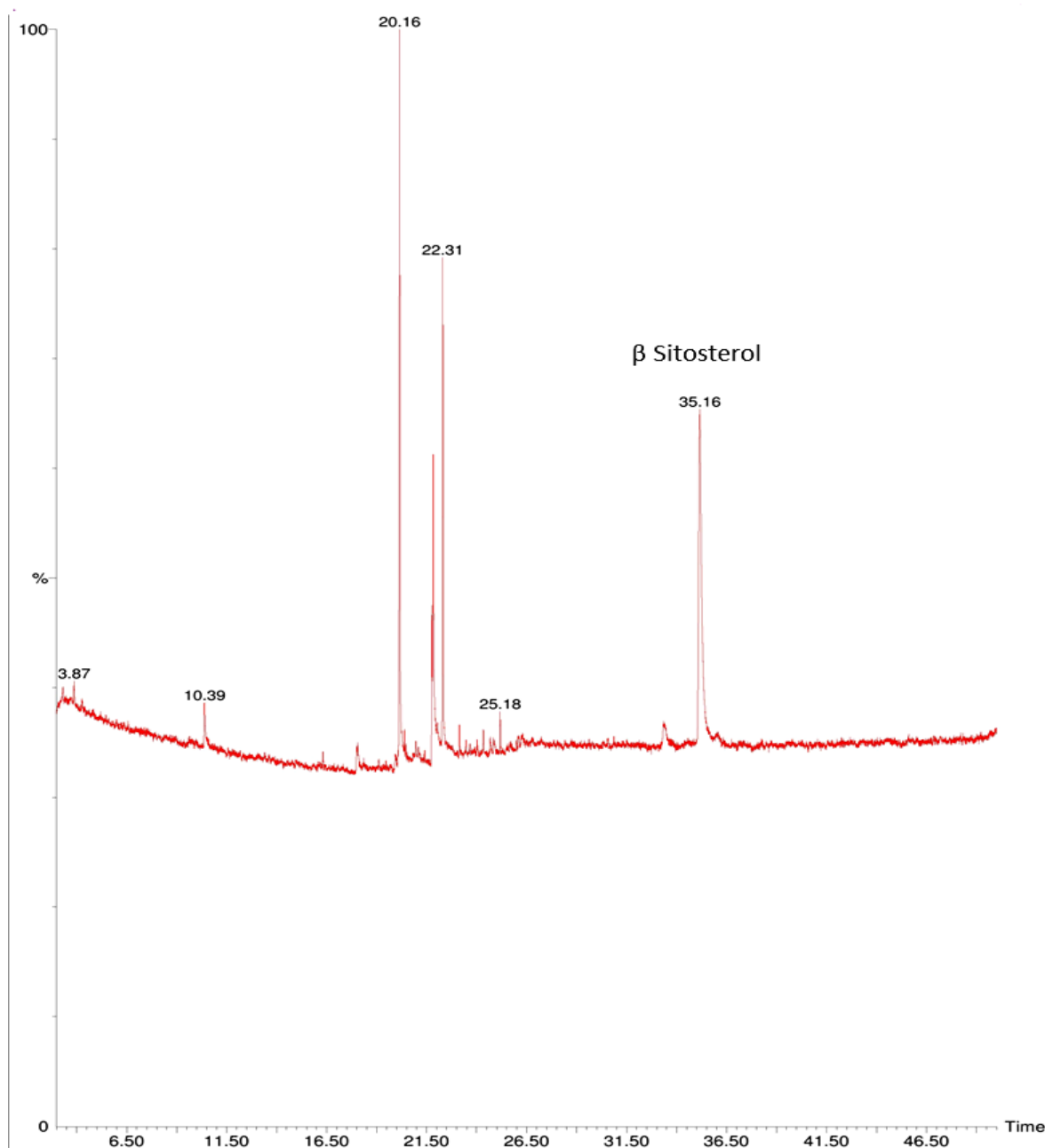


Figure 6.3.12 Mass spectra of β Sitosterol separated in GC chromatogram at RT 35.16 min in the P_1 fraction

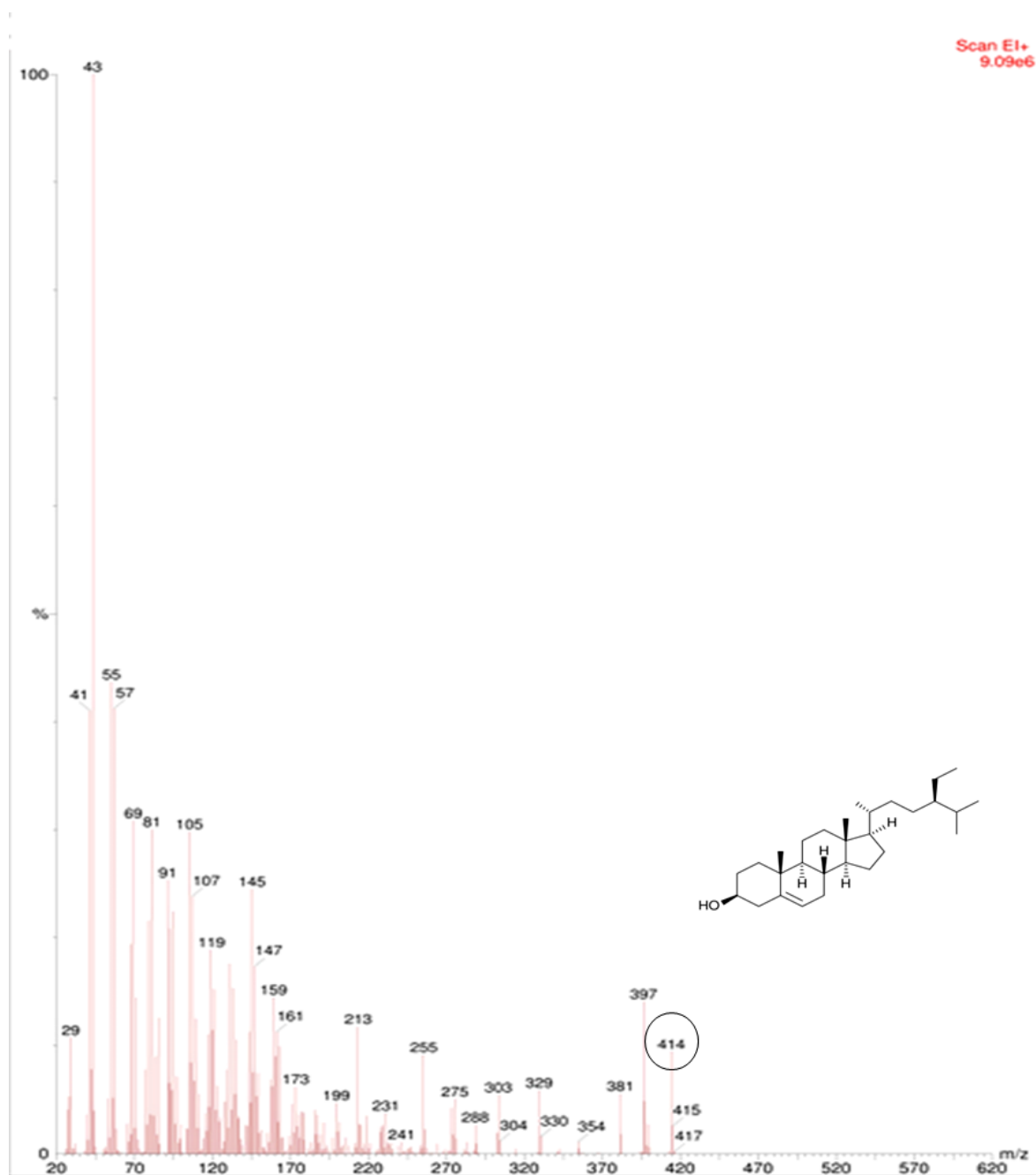


Table 6.3.9 The detailed information on the other probable phytochemical constituents detected in the P₁ fraction of *Aloe vera* gel

No.	Compound Name	Formula	Molecular Weight
1	3-ACETOXY-BISNOR-5-CHOLENIC ACID	C ₂₄ H ₃₆ O ₄	388
2	20-ETHYNYL-5-PREGNEN-3,20-DIOL	C ₂₃ H ₃₄ O ₂	342
3	CHOLESTA-3,5-DIENE	C ₂₇ H ₄₄	368
4	26-NOR-5-CHOLESTEN-3.BETA.-OL-25-ONE	C ₂₆ H ₄₂ O ₂	386
5	26-NOR-5-CHOLESTEN-3.BETA.-OL-25-ONE	C ₂₆ H ₄₂ O ₂	386
6	STIGMASTAN-3,5-DIENE	C ₂₉ H ₄₈	396
7	BETA- SITOSTEROL	C ₂₉ H ₅₀ O	414
8	GAMMA.-SITOSTEROL	C ₂₉ H ₅₀ O	414
9	CHOLEST-5-EN-3-OL (3.BETA.)-, ACETATE	C ₂₉ H ₄₈ O ₂	428
10	CHOLEST-8-EN-3.BETA.-OL, ACETATE	C ₂₉ H ₄₈ O ₂	428
11	CHOLEST-5-EN-3-OL (3.BETA.)-, ACETATE	C ₂₉ H ₄₈ O ₂	428
12	CHOLEST-5-EN-3-OL (3.BETA.)-, PROPANOATE	C ₃₀ H ₅₀ O ₂	442
13	CHOLEST-5-EN-3-OL (3.BETA.)-, PROPANOATE	C ₃₀ H ₅₀ O ₂	442
14	CHOLEST-5-EN-3-OL (3.BETA.)-, CARBONCHLORIDATE	C ₂₈ H ₄₅ O ₂ Cl	448
15	BETA.-SITOSTEROL ACETATE	C ₃₁ H ₅₂ O ₂	456
16	BETA.-SITOSTEROL ACETATE	C ₃₁ H ₅₂ O ₂	456
17	CHOLEST-5-EN-3-OL (3.BETA.)-, TETRADECANOATE	C ₄₁ H ₇₂ O ₂	596
18	22, 23-DIBROMOSTIGMASTEROL ACETATE	C ₁₃ H ₅₀ O ₂ Br ₂	612

Apart from β sitosterol, various phytochemical constituents have been detected during the GC MS/MS analysis of the P₁ fraction of *Aloe vera* gel. These compounds were similar to cholesterol structure and soluble in petroleum ether. These phytochemical constituents detected could also play major role in the ovarian modulation as indicated in *ex vivo* experiment.

6.4 DISCUSSION

Aloe vera is well known medicinal plant for several biological properties (Radha and Laxmipriya 2015). The gel is rich in phytonutrients and reported to have various medicinal properties (Reynolds, 1990). Aim of current chapter was to explore its phytochemical analysis *Aloe vera* gel through different phytochemical analysis that included qualitative and quantitative analysis confirming the presence of phytocomponents namely, polysaccharides, alkaloids, polyphenols, sterols, flavonoids, anthraquinones etc. and identification of active phytocomponents which may play a major role in ovarian modulation (Hamman 2008). Our earlier chapter was to investigate the effect of *Aloe vera* gel in management of PCOS rat model wherein *Aloe vera* gel helped to normalize the PCO phenotype and support for successful pregnancy (Maharjan et al. 2010; Radha et al. 2014). Hence, an “*ex vivo*” experiment was done wherein partially purified fractions was incubated with polycystic ovarian tissue to check direct effect on steroidogenesis. Results favored our hypothesis wherein petroleum ether extracted P1 fraction demonstrated significant change in ovarian steroidogenic enzyme activity compared to other fractions. From literature, reports also suggested that phytosterols have modulatory effect on key protein StAR in ovarian steroidogenesis (Sharpe et al. 2007). It has estrogenic potentiality which able to mimic estrogen receptor (ER) binding and modulate the function (Cotroneo et al. 2001). Thereby, further aim was to investigate detailed composition of phytocomponents of petroleum ether fraction. (P1). In qualitative fingerprint of TLC, P1 fraction exhibited presence of various bands of phytosterols that matched with respective standards. Hence, further quantified these phytocomponents with more sensitive methods- HPTLC and HPLC, wherein, β sitosterol exhibited maximum amount of the content out of other phytosterols. It is also known that phytosterols may potentiate the ovarian function. Thereby, GC analysis was performed. The results suggested the presence of several phytosterols and its derivatives in addition to stigmasterol, sitosterol and lupeol.

6.5 CONCLUSION

Aloe vera gel is rich in several phytocomponents that were quantified by a simple, rapid, reproducible, and sensitive HPTLC method and method was validated using Lupeol as a standard. Method was successfully validated as per ICH guidelines and statistical analysis proved that the method is sensitive, specific, and repeatable. Further, confirmation of Phytosterols like stigmasterol, β - sitosterol, lupeol etc has been performed by HPLC. Further, GC MS analysis confirmed the presence of these Phytosterols, which may act as modulator of ovarian function. This fact could be justified by understanding its efficacy “*in vivo*”.

6.6 REFERENCES

- Ashiru O. 2006. Pregnancy outcome following swim up preparation of both fresh and cryopreserved spermatozoa.
- Bankole MA, Shittu LA, Ahmed TA, Bankole MN, Shittu RK, Terkula K, Ashiru OA. 2008. Synergistic antimicrobial activities of phytoestrogens in crude extracts of two sesame species against some common pathogenic microorganisms. *African Journal of Traditional, Complementary and Alternative Medicines* **4**: 427-433.
- Cotroneo MS, Wang J, Eltoum I-EA, Lamartiniere CA. 2001. Sex steroid receptor regulation by genistein in the prepubertal rat uterus. *Molecular and cellular endocrinology* **173**: 135-145.
- Hamman JH. 2008. Composition and applications of Aloe vera leaf gel. *Molecules* **13**: 1599-1616.
- Kim K, Kim H, Kwon J, Lee S, Kong H, Im S-A, Lee Y-H, Lee Y-R, Oh S-T, Jo TH. 2009. Hypoglycemic and hypolipidemic effects of processed *Aloe vera* gel in a mouse model of non-insulin-dependent diabetes mellitus. *Phytomedicine* **16**: 856-863.
- Maharjan R, Nagar PS, Nampoothiri L. 2010. Effect of *Aloe barbadensis* Mill. formulation on Letrozole induced polycystic ovarian syndrome rat model. *Journal of Ayurveda and integrative medicine* **1**: 273.
- Mellanen P, Petänen T, Lehtimäki J, Mäkelä S, Bylund G, Holmbom B, Mannila E, Oikari A, Santti R. 1996. Wood-derived estrogens: studies in vitro with breast cancer cell lines and in vivo in trout. *Toxicology and applied pharmacology* **136**: 381-388.
- Nascimento GG, Locatelli J, Freitas PC, Silva GL. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian journal of microbiology* **31**: 247-256.
- Pérez YY, Jiménez-Ferrer E, Zamilpa A, Hernández-Valencia M, Alarcón-Aguilar FJ, Tortoriello J, Román-Ramos R. 2007. Effect of a polyphenol-rich extract from *Aloe vera* gel on experimentally induced insulin resistance in mice. *The American journal of Chinese medicine* **35**: 1037-1046.
- Prabjone R, Thong-Ngam D, Wisedopas N, Chatsuwan T, Patumraj S. 2006. Anti-inflammatory effects of *Aloe vera* on leukocyte-endothelium interaction in the gastric microcirculation of Helicobacter pylori-infected rats. *Clinical hemorheology and microcirculation* **35**: 359-366.

- Radha M, Padamnabhi N, Laxmipriya N. 2014. Evaluation of *Aloe barbadensis mill.* Gel on letrozole induced polycystic ovarian syndrome (pcos) rat model-a dose dependent study. *International Journal of Pharmaceutical Sciences and Research* **5**: 5293.
- Radha MH, Laxmipriya NP. 2015. Evaluation of biological properties and clinical effectiveness of *Aloe vera*: A systematic review. *Journal of Traditional and Complementary Medicine* **5**: 21-26.
- Rajasekaran S, Ravi K, Sivagnanam K, Subramanian S. 2006. Beneficial effects of Aloe vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes. *Clinical and Experimental Pharmacology and Physiology* **33**: 232-237.
- Rajasekaran S, Sivagnanam K, Ravi K, Subramanian S. 2004. Hypoglycemic effect of Aloe vera gel on streptozotocin-induced diabetes in experimental rats. *Journal of Medicinal food* **7**: 61-66.
- Rosenblum E, Stauber R, Thiel Dv, Campbell I, Gavalier J. 1993. Assessment of the estrogenic activity of phytoestrogens isolated from bourbon and beer. *Alcoholism: Clinical and Experimental Research* **17**: 1207-1209.
- Sharpe RL, Woodhouse A, Moon TW, Trudeau VL, MacLachy DL. 2007. β -Sitosterol and 17 β -estradiol alter gonadal steroidogenic acute regulatory protein (StAR) expression in goldfish, *Carassius auratus*. *General and comparative endocrinology* **151**: 34-41.
- Shittu L. 2010. Reproductive impact of sesame leaves lignans in adult male SD rats. *LAP Lambert Academic Publishing ISBN*: 978-973.
- Shittu L, Shittu R, Olufemi O, Tayo A, Osunubi A. 2009. Hypoglycaemia and improved testicular parameters in *Sesamum radiatum* treated normo-glycaemic adult male Sprague Dawley rats. *African Journal of Biotechnology* **8**.
- Tamura N, Yoshida T, Miyaji K, Sugita-Konishi Y, Hattori M. 2009. Inhibition of infectious diseases by components from Aloe vera. *Bioscience, biotechnology, and biochemistry* **73**: 950-953.
- Yongchaiyudha S, Rungpitarangsi V, Bunyaphrathatsara N, Chokechaijaroenporn O. 1996. Antidiabetic activity of Aloe vera L. juice. I. Clinical trial in new cases of diabetes mellitus. *Phytomedicine* **3**: 241-243.