CHAPTER 5

5. Preliminary Screening of Extracts for PL Inhibition

Traditional medicines are referred to those drugs that are obtained either from single medicinal plant or combination of many such plants. Preliminary screening of these medicinal plants is the first and most important stage of drug discovery against a certain disease. Drug discovery is a process which requires huge amount of resources and time. Thus, this stage of drug discovery plays a vital role in the maintenance of the above said parameters. There are various *in vitro* assays that have been developed to mimic the human disease conditions to speed up the identification of drug leads and to reduce the large requirement of animals for preclinical studies. Such assays are used to evaluate and rank a large number of plant extracts in a high-throughput manner. The extracts that produced potent inhibition in the screening step are further selected for more comprehensive studies [1].

5.1 PL inhibition by plant extracts

Based on literature review, 17 medicinal plants having anti-obesity use in traditional system of medicine were selected. One hundred and fifty-three crude extracts were prepared using selected medicinal plants (Section 4.1 & 4.3). The inhibitory activities towards PL were evaluated and the IC_{50} of these extracts are reported in **Table 5** & **6**. Out of One hundred and fifty-three extracts, only nine extracts exhibited $IC_{50} \leq 20$ μg/ml. These extracts included TAM-SO, CZW-SO, OGM-SO, GSM-SO, AVH-SX, BARM-SO, AMM-SO, PLM-SO, and TSM-SO.

Table 5: PL inhibition of selected plants using different extraction techniques and organic solvents

Sl no.	Plant (parts)	Solvent	Code	IC_{50} (μg/ml)		
				Sonication (SO)	Soxhlation (SX)	Maceration (MC)
1	*Ocimum gratissimum* (leaves)	Hexane	OGH	33.89 ± 0.44	21.29 ± 0.781	23.63 ± 0.33
2		Methanol	OGM	7.76 ± 0.37	19.93 ± 0.74	15.10 ± 0.64
3	*Cinnamomum zeylanicum* (bark)	Hexane	CZH	38.01 ± 0.97	32.6 ± 0.57	40.79 ± 1.89
4		Methanol	CZM	21.35 ± 0.47	14.07 ± 0.15	23.54 ± 0.46
5	*Terminalia arjuna* (bark)	Hexane	TAH	36.57 ± 1.35	21.59 ± 0.36	56.26 ± 4.46
6		Methanol	TAM	1.64 ± 0. 49	17.469 ± 0.20	34.04 ± 0.70
7	*Azadirachta indica* (leaves)	Hexane	AILH	41.68 ±1.91	51.25 ± 1.23	84.85 ± 0.46
8		Methanol	AILM	28.28 ±0.76	31.09 ± 0.27	55.24 ±0.98
9	*Aloe vera* (leaves)	Hexane	AVH	18.18 ± 0.24	9.57 ± 0.29	50.56 ± 4.67
10		Methanol	AVM	32.14 ± 0.13	28.61 ± 1.06	33.68 ± 0.83

Sl no	Plant (parts)	Solvent	Code	IC_{50} (µg/ml)		
				Sonication (SO)	Soxhlation (SX)	Maceration (MC)
11	*Berberis aristata* (roots)	Hexane	BARH	21.37 ± 0.80	26.26 ± 0.61	32.88 ± 0.86
12		Methanol	BARM	11.10 ± 1.24	31.86 ± 5.94	44.81 ± 5.20
13	*Zingiber officinale* (rhizomes)	Hexane	ZOH	64.10 ± 0.44	76.41± 1.14	68.17 ± 0.87
14		Methanol	ZOM	39.81 ± 0.91	26.45 ± 0.98	25.68 ± 1.10
15	*Piper nigrum* (fruits)	Hexane	PNH	31.16 ± 2.40	38.76 ± 1.16	47.84 ± 2.64
16		Methanol	PNM	21.23 ± 0.44	29.67 ± 0.49	23.75 ± 1.73
17	*Piper chaba* (fruits)	Hexane	PChH	42.74 ± 0.69	33.11 ± 0.92	51.99 ± 6.03
18		Methanol	PChM	25.85 ± 2.05	31.10 ± 1.94	45.12 ± 1.40
19	*Piper longum* (fruits)	Hexane	PLH	51.40 ± 1.12	42.97 ± 1.47	99.87 ± 1.28
20		Methanol	PLM	14.10 ± 0.70	26.30 ± 0.96	56.49 ± 3.98
21	*Cleodendrum serratum* (leaves)	Hexane	CSH	35.63 ± 1.22	35.48 ± 0.18	49.05 ± 5.20
22		Methanol	CSM	28.59 ± 0.31	28.69 ± 1.65	49.86 ± 4.04
23	*Gymnema sylvestre* (leaves)	Hexane	GSH	31.75 ± 0.88	56.29 ± 4.60	83.53 ± 1.18
24		Methanol	GSM	7.91 ± 1.18	9.82 ± 1.44	21.40 ± 2.46
25	*Aegle marmelos* (fruits)	Hexane	AMH	29.23 ± 0.75	35.35 ± 2.52	35.13 ± 1.94
26		Methanol	AMM	13.02 ± 2.05	43.58 ± 1.98	37.15 ± 5.00
27	*Sphaeranthus indicus* (bark)	Hexane	SIH	31.43 ± 1.54	34.09 ± 0.56	39.07 ± 0.97
28		Methanol	SIM	23.99 ± 0.60	21.40 ± 0.98	31.78 ± 0.45
29	*Sympolocus racemosa* (bark)	Hexane	SRH	34.66 ± 0.07	32.12 ± 0.43	48.09 ± 1.87
30		Methanol	SRM	28.37 ± 0.35	34.75 ± 1.64	39.06 ± 0.56
31	*Emblica officinalis* (fruits)	Hexane	EOH	32.87 ± 3.21	49.91 ± 5.03	32.37 ± 3.37
32		Methanol	EOM	49.97 ± 3.92	55.45 ± 5.24	64.77 ± 5.01
33	*Thea sinensis* (leaves)	Hexane	TSH	27.80±1.02	31.66±1.06	52.47±1.47
34		Methanol	TSM	20.00±1.16	21.49±1.21	28.77±1.04

All experiments were performed in triplicate (n = 3). All the values are expressed as mean± S.E.M.

Table 6: PL inhibition of selected plants using different extraction technique and water as solvent

Sl no.	Plant (parts)	Code	IC_{50} (µg/ml)		
			Sonication (SO)	Decoction (DC)	Maceration (MC)
1	*Terminalia arjuna* (bark)	TAW	12.14 ± 1.63	18.46 ± 0.24	24.47 ± 0.63
2	*Ocimum gratissimum* (leaves)	OGW	18.42 ± 1.22	32.44 ± 0.20	57.81 ± 0.74
3	*Gymnema sylvestre* (leaves)	GSW	8.80 ± 0.30	9.23 ± 0.41	11.89 ± 0.53
4	*Berberis aristata* (roots)	BARW	42.70 ± 1.41	49.04 ± 0.45	74.95 ± 4.53
5	*Zingiber officinale* (rhizomes)	ZOW	67.23 ± 0.23	58.14 ± 0.98	69.77 ±0.54

Sl no.	Plant (parts)	Code	IC_{50} (µg/ml)		
			Sonication (SO)	Decoction (DC)	Maceration (MC)
6	*Aegle marmelos* (fruits)	AMW	38.86 ± 0.53	26.30 ± 0.86	67.33 ± 0.41
7	*Piper longum* (fruits)	PLW	47.66 ± 0.44	53.37 ± 0.35	65.19 ± 0.72
8	*Cinnamomum zeylanicum* (bark)	CZW	1.89 ± 0.53	2.92 ± 0.47	4.34 ± 0.09
9	*Azadirachta indica* (leaves)	AILW	89.93 ± 0.54	87.92 ± 0.71	97.61 ± 0.20
10	*Cleodendrum serratum* (leaves)	CSW	47.04 ± 0.24	49.78 ± 0.06	54.11 ± 0.98
11	*Piper nigrum* (fruits)	PNW	53.09 ± 0.34	57.33 ± 0.67	61.45 ± 0.12
12	*Piper chaba* (fruits)	PChW	34.91 ± 0.41	35.76 ± 0.65	48. 54 ± 0.34
13	*Aloe vera* (leaves)	AVW	37.23 ± 0.23	38.14 ± 0.98	39.77 ±0.54
14	*Sphaeranthus indicus* (bark)	SIW	49.01 ± 0.43	47.20 ± 0.77	62.67 ± 1.76
15	*Sympolocus racemose* (bark)	SRW	39.87 ± 0.56	42.98 ± 0.98	48.00 ± 1.67
16	*Emblica officinalis* (fruits)	EOW	40.61 ± 0.74	43.32 ± 0.32	74.48 ± 0.62
17	*Thea sinensis* (leaves)	TSW	27.55 ± 0.12	32.46 ± 0.89	40.21 ± 0.34

All experiments were performed in triplicate (n = 3). All the values are expressed as mean± S.E.M.

Orlistat was used as standard and all the results were compared with Orlistat that exhibited PL inhibition with IC_{50} of 0.49 µg/ml. There are various possible reasons to justify the potent enzyme inhibition of the above plant extracts. Chemical constituents being most important factor followed by the extract technique and solvent being used. The chemical constituents of these medicinal plants have the following properties such as large molecular volume for their ability to interact with the active site and presence of an ester group with a reactive carbonyl functional group that would interact with Ser152 of the active site [2,3].

Extraction of the crude plant material is a crucial step prior to their bio-evaluation. The most common extraction techniques used in laboratory are hot percolation (soxhlation), ultrasonication, and cold percolation (maceration) [4]. In all the extraction techniques, an important parameter is the type of solvent used. Non-polar solvents (e.g., hexane or petroleum ether) extracts sterols, terpenes; while, polar solvents such as methanol or ethanol help in extracting out polar compounds including alkaloids and polyphenols [5]. Majority of research articles do not compare and correlate the biological efficacy of plant extracts with their extraction procedures. On the other hand, they mostly report the use of any one of the above techniques for preparation and bio-evaluation, which do not necessarily represent the true scenario. Thus, contextual optimization is needed to precisely understand the best extraction

procedure to obtain the most effective biological results [6]. The present study evaluated the PL inhibition potential of various extracts. The study also highlighted the comparison and correlation of the observed biological efficacy of above plant extracts with type of extraction techniques used.

The amount of chemical constituents present in the extract is also an important criterion for improved potency. This criterion can be explained by the extraction technique and the solvents used. Ultrasonication was performed in an ultrasonic bath with 100 W power and in a continuous mode at a frequency of 40 kHz (LMUC-4, Labman Scientific Instruments, India) [7]. Soxhlation was performed for 24 h in which the solvent was heated, evaporated and condensed back into the closed soxhlet apparatus as a continuous process. On the other side in maceration crude drug was soaked in solvent for 72 h with intermittent stirring after every 12 h. Hence, ultrasonication and soxhlation extraction were probably were capable to extract active constituents into the solvents [8]. As Ultrasonication consumes 100W power with continuous frequency of 40 kHz, it has the capability to rupture the cell wall and membrane of the plant material to extract out the maximum chemical constitutents present in it[9].Thus, this becomes a major advantage of ultrasonication compared to soxhalation. However, solvent type is also a significant factor to obtain more desired chemical constituents [6]. From the above results it can be concluded that methanol extracts were more potent compared to hexane extracts. This was due to the capacity of methanol to extract out polar compounds containing reactive functionlaity [10,11].

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