Contents

Ρ	Prefac	ce N	/ii
C	Colou	r Plates Between pages 86 and 8	37
1.	Intro	oduction to Experimental Pharmacology	1
2.	In vi	itro Anti-inflammatory Assay	14
	2.1	To study the <i>in vitro</i> anti-inflammatory potential of the given sample against heat-induced protein denaturation <i>17</i>	
	2.2	To study the <i>in vitro</i> anti-inflammatory potential of the given sample by membrane stabilization assay (heat-induced hemolysis) <i>19</i>	
	2.3	To study the <i>in vitro</i> anti-inflammatory potential of the given sample by membrane stabilization assay (hypotonicity-induced hemolysis) <i>21</i>	
	2.4	To study the <i>in vitro</i> 5-lipoxygenase inhibitory potential of the given sample using 5-lipoxygenase inhibition assay 23	
	2.5	To study the <i>in vitro</i> hyaluronidase inhibitory potential of the given sample using hyaluronidase inhibition assay 25	
	2.6	To study the <i>in vitro</i> anti-inflammatory potential of the given sample by COX inhibition using luminescent assay protocol <i>29</i>	
	2.7	To study the <i>in vitro</i> COX inhibition potential of the given sample by using oxygen uptake assay protocol <i>32</i>	
	2.8	To study the <i>in vitro</i> COX inhibition potential of the given sample by using peroxidase assay <i>35</i>	
	2.9	To study the <i>in vitro</i> COX inhibition potential of the given sample by using prostaglandin E2 enzyme-linked immunosorbent assay (PGE2 ELISA) <i>37</i>	
3.	In vi	itro Antioxidant Assay	41
	3.1	To study the <i>in vitro</i> antioxidant potential of the given sample by DPPH scavenging activity <i>42</i>	
	3.2	To study the <i>in vitro</i> anti-inflammatory potential of the given sample by hydrogen peroxide scavenging $(H_2O_2)$ assay 44	

- 3.3 To study the *in vitro* antioxidant potential of the given sample by nitric oxide scavenging activity 46
- 3.4 To study the *in vitro* antioxidant potential of the given sample by reducing power method 48
- 3.5 To study the *in vitro* antioxidant potential of the given sample by total antioxidant capacity assay (phosphomolybdenum method) *50*
- 3.6 To study the *in vitro* antioxidant potential of the given sample by ferric reducing antioxidant power (FRAP) assay *52*
- 3.7 To study the *in vitro* antioxidant potential of the given sample by xanthine oxidase activity *54*
- 3.8 To study the *in vitro* antioxidant potential of the given sample by hydroxyl (HO') radical scavenging assay 56
- 3.9 To study the *in vitro* antioxidant potential of the given sample by superoxide radical scavenging assay *58*

## 4. In vitro Antidiabetic Assay

- 4.1 To study the *in vitro* antidiabetic potential of the given sample by determining its  $\alpha$ -amylase inhibitory action using starch iodine method 63
- 4.2 To study the *in vitro* antidiabetic potential of the given sample by  $\alpha$ -glucosidase inhibitory assay 65
- 4.3 To study the *in vitro* antidiabetic potential of the given sample by glucose diffusion assay *67*
- 4.4 To study the *in vitro* antidiabetic potential of the given sample by protein tyrosine phosphatase inhibition assay 69
- 4.5 To study the *in vitro* antidiabetic potential of the given sample by glycation inhibition assay 71

## 5. In vitro Enzyme-Based Assay

- 5.1 To study the *in vitro* protease inhibitory potential of the given sample using protease inhibition assay *75*
- 5.2 To study the *in vitro* tyrosinase inhibitory potential of the given sample using tyrosinase inhibition assay *78*
- 5.3 To study the *in vitro* acetylcholinesterases (AChE) inhibitory potential of the given sample using acetylcholinesterases inhibition assay *80*

## 6. In vitro Antimicrobial Assay

6.1 To study the antimicrobial sensitivity of the given sample using Kirby-Bauer method 94

74

60

82

	Contents	xi
6.2	To study the antimicrobial sensitivity of the given sample using Stokes method <i>100</i>	
6.3	To determine the minimum inhibitory concentration (MIC) of the given sample using broth dilution method <i>103</i>	
6.4	To determine the minimum inhibitory concentration (MIC) of the given sample using agar dilution method <i>106</i>	
6.5	To determine the minimum bactericidal concentration (MBC) of the given sample using broth dilution test <i>108</i>	
6.6	To study the <i>in vitro</i> $\beta$ -lactamase inhibitory potential of the given sample using $\beta$ -lactamase inhibition assay 110	
7. In vi	itro Assay for Tropical Disease	112
7.1	To study the <i>in vitro</i> antimalarial activity of the given sample by using 72 hours <i>in vitro</i> growth inhibition assay <i>131</i>	
7.2	To study the <i>in vitro</i> antimalarial activity of the given sample by using hemozoin-based colorimetric method 133	
7.3	To study the <i>in vitro</i> antimalarial activity of the given sample by using candle jar method <i>136</i>	
7.4	To study the <i>in vitro</i> activity of the given sample against leishmaniasis using leishmanicidal assay 138	
8. In vi	itro Anticancer Assay	140
8.1	To study the <i>in vitro</i> antimitotic potential of the given sample using <i>Allium cepa</i> root tip assay 143	
8.2	To study the <i>in vitro</i> antimitotic potential of the the given sample using germination assay 146	
8.3	To study the in vitro MTS cytotoxicity assays 150	
8.4	To study the in vitro MTT cytotoxicity assays 152	
8.5	To study the <i>in vitro</i> apoptosis by estimation of DNA fragmentation <i>154</i>	
8.6	To study annexin A5-induced apoptosis (annexin A5 affinity assay) by flow cytometery <i>159</i>	
8.7	To study annexin A5-induced apoptosis (annexin A5 affinity assay) by confocal scanning-laser microscopy (CSLM) <i>163</i>	
8.8	To study in vitro apoptosis by TUNEL assay 166	
8.9	To study the antiangiogenic activity of the given sample by chorioallantoic membrane assay (CAM) 177	
8.10	To study the antiangiogenic activity of the given sample by endothelial cell tube formation assay 180	

Index

187