

## CHAPTER 4

### MATERIALS AND METHODS

#### 4.1 PLANT MATERIAL

Fruits of *L. acidissima* were purchased from local market of Chennai in the month of August 2007. The fruit was authenticated by Prof P. Jayaraman, taxonomist at Plant Anatomy Research Centre (PARC), Tambaram, Chennai. A voucher specimen (SRMCP/08/07) was deposited in the Department of Pharmacognosy, SRM College of Pharmacy, SRM University for future reference. The fruit was shade dried, separated mechanically into fruit pulp and shell then coarsely powdered using a cutter mill and stored in an air-tight, light resistant container for further use.

#### 4.2 EXPERIMENTAL ANIMALS

Adult albino male rats of wistar strain weighing 150 - 175 g were used in the pharmacological and toxicological studies. The experimental animals were maintained in a well ventilated animal house, Department of Pharmacology, SRM College of Pharmacy, SRM University, which is maintained at a temperature between 20-25<sup>0</sup>C and humidity between 30-70%. The animals were exposed to 12 h light and 12 h dark lighting condition controlled by automatic timer. They were kept in standard polypropylene rat cages with stainless steel top grill supplied by Inco, India, with sterilized paddy husk as the bedding material. The animals were fed with balanced rodent pellet diet from Poultry Research Station, Nandanam, Chennai, India and tap water was supplied throughout the experimental period. The drinking

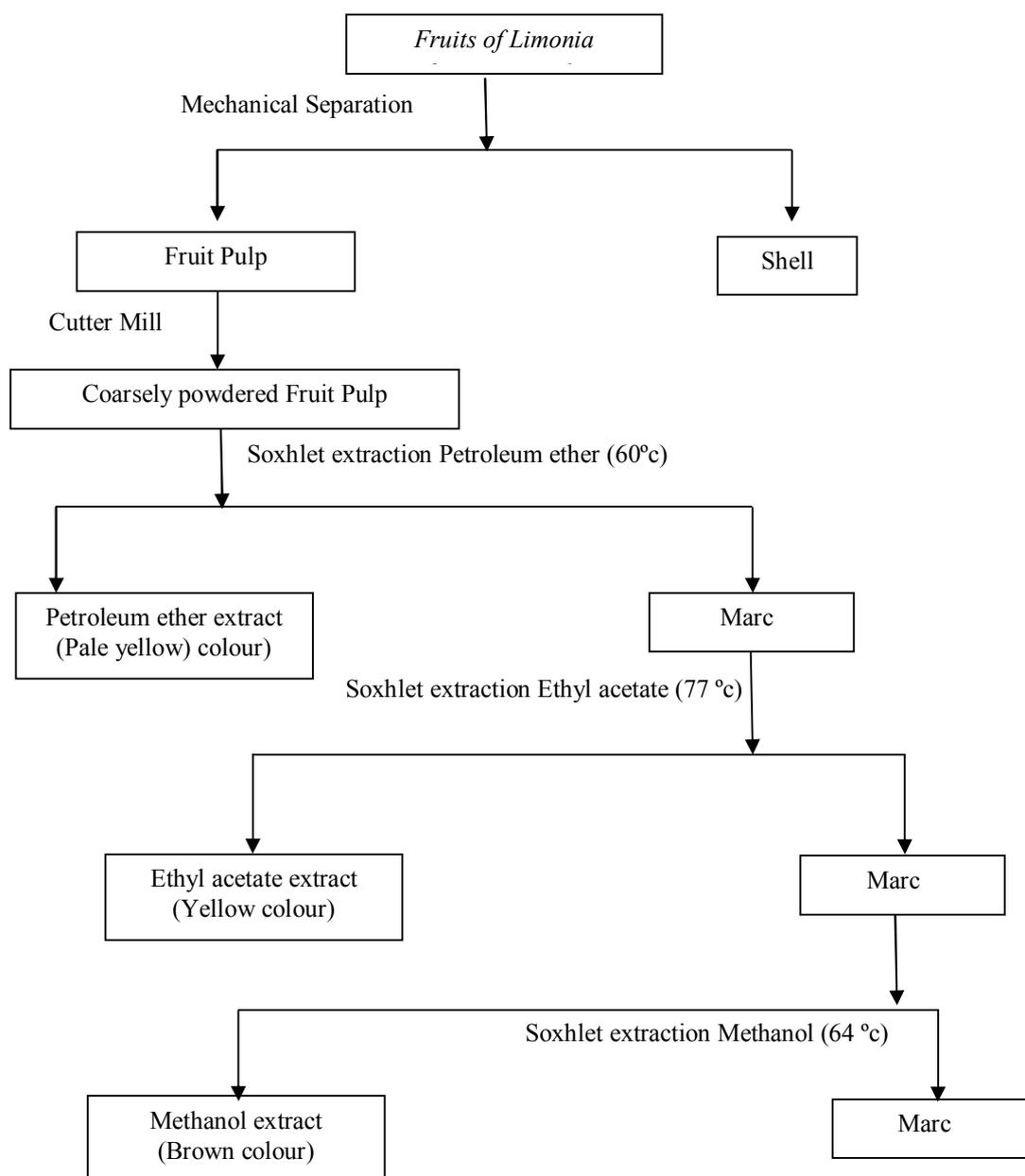
water and the feed were provided *ad libitum*, except during fasting. The animals were housed 10 days prior to the experiment to acclimatize to the laboratory conditions. Prior to the study, the experimental protocol was submitted to the CPCSEA / IAEC and got approved and the Approval number being IAEC/30/2007.

### **4.3 PREPARATION OF EXTRACT**

The coarsely powdered, shade dried fruit pulp was first defatted with Petroleum ether using soxhlet apparatus. The extract was concentrated using rotary evaporator to get solid residue. The marc from the central compartment was removed, dried and extracted by exhaustive extraction with a series of solvents of increasing polarity with Soxhlet extractor was done. Solvents used with increasing polarity are petroleum ether, ethyl acetate, methanol and the scheme of extraction was depicted as a flow chart (Figure 4.1) below.

#### **4.3.1 Scheme of Extraction**

The percentage yield of each extracts was calculated on dry weight analysis and it was found to be 0.01% w/w for Petroleum ether, 0.02% w/w for chloroform, 0.07% w/w for ethyl acetate and 23.6% w/w for methanol. Since the yield obtained from the fruit pulp in methanol was quantifiable than the other extracts, the methanolic extract was then stored in a desiccators for further phytochemical and pharmacological studies. The results are depicted in Table 4.1.



**Figure 4.1** Scheme of extraction for the Fruit pulp of *Limonia acidissima*

#### 4.4 PRELIMINARY PHYTOCHEMICAL SCREENING

The prepared methanolic extract was subjected to routine phytochemical analysis [69] to identify the presence of various phytochemicals such as carbohydrates, alkaloids, glycosides, saponins, flavonoids, tannins, sterols, phenols, etc. The results are depicted in Table 4.2.

#### 4.5 RESULTS

**Table 4.1 Extractive value of the extracts**

S.No	Extracts	Colour and Consistency	Extractive value in %w/w
1.	Pet. Ether	Green/ Sticky mass	0.01
2.	Ethyl acetate	Yellowish green/ Sticky mass	0.02
3.	Methanol	Brownish green/ Thick solid mass	23.6

**Table 4.2 Preliminary phytochemical screening**

Sl.No	Plant constituents and Tests	Methanolic extract
1.	<b>Test for Carbohydrates</b>	
	a.Molisch's test	+
	b.Fehling's test	+
	c.Benedict's test	+
	d.Barfoed's test	+
	e.Test for Starch	-
2.	<b>Test for Gums and Mucilages</b>	
	a. Alcoholic precipitation	-
	b. Molisch's test	+

Sl.No	Plant constituents and Tests	Methanolic extract
3.	<b>Test for Proteins and Amino acids</b>	
	a.Ninhydrin test	+
	b.Biuret test	+
	c.Millon's test	+
	d.Zanthoproteic test	+
	e.Tannic acid test	+
4.	<b>Test for Fixed oils and Fats</b>	
	a.Spot test	-
	b.Saponification test	-
5.	<b>Test for Alkaloids</b>	
	a.Mayer's test	-
	b.Dragendorff's test	-
	c.Wagner's test	-
	d.Hager's test	-
6.	<b>Test for Saponins</b>	
	a.Frothing test	-
7.	<b>Test for Glycosides</b>	
	a.Legal's test	+
	b.Baljet's test	+
	c.Borntrager's test	+
	d.Keller-kiliani test	+
	e.Terpenoid glycoside test	+
8.	<b>Test for Phytosterols</b>	
	a.Libermann's test	-
	b.Libermann Burchard test	-
	c.Salkowski's test	-

<b>Sl.No</b>	<b>Plant constituents and Tests</b>	<b>Methanolic extract</b>
9.	<b>Test for Flavonoids</b>	
	a. Ferric chloride test	+
	b. Alkaline- reagent test	+
	c. Zinc-hydrochloride reduction test	+
	d. Lead acetate solution test	+
	e. Mineral acid reaction test	+
	f. Boric acid test	+
10.	<b>Test for Tannins and Phenolic compounds</b>	
	a. Reaction with copper sulphate	+
	b. Ferric chloride (5%) test	+
	c. Reaction with Lead acetate	+
	d. Reaction with Potassium dichromate	+
	e. Reaction with Potassium ferricyanide	+
	f. Gelatin test	+

+ ⇨ **Presence of components**

- ⇨ **Absence of components**