LIST OF CONTENTS

Chapte	r	Page
I	INTRODUCTION	. 1
	Rationale for the study	
	Main objective of the study	
	Specific objectives of the study	. 3
	The scope of the study	
	Hypothesis	
	Research place	
	Research plans	
п	REVIEW OF RELATED LITERATURE AND RESEARCH	6
	Asparagus racemosus Willd	6
	Pharmacological properties of AR root	
	Phytoestrogens	15
	Menopause	16
	Bone loss	
	Plants, medicinal herbs and herbal supplements of osteoporosis	24
	Uterus	28
	Mammary gland	30
	Animal model of osteoporosis	
III	RESEARCH METHODOLOGY	33
	Instruments and materials	33
	Methods	24

LIST OF CONTENTS (CONT.)

Chapte	er.	Page
IV	RESULTS	. 41
	Effect of AR root extract on femoral parameters	
	Effect of AR root extract on biochemical parameters	
	Effect of AR root extract on calcium, phosphorus, ALP and	
	estradiol level	. 45
	Histological analysis of femur	
	Histomorphometric analysis of trabecular bone	. 51
	Histological analysis of cortical bone	
	Histomorphometric analysis of cortical bone thickness	
	Body weight	
	Uterine weight	
	Histological analysis of uterus	61
	Histomorphometric analysis of uterus	65
	Histological analysis of mammary gland	
	Histomorphometric analysis of mammary gland	
\mathbf{V}	DISCUSSIONS	. 71
	Effect of AR root extract on femoral parameters	
	Effect of AR root extract on bone markers	72
	Effect of AR root extract on calcium, phosphorus, ALP and	
	estradiol level	73
	Effect of AR root extract on femoral histology and	
	histomorphometry	74
	Effect of AR root extract on body weight	
	Effect of AR root extract on reproductive organs	
VI	CONCLUSIONS	77

LIST OF CONTENTS (CONT.)

Chapter	Page
REFERENCES	78
APPENDIX	94
BIOGRAPHY	97



LIST OF TABLES

Tab	le	Page
1	Research Dlans	
2	Research Plans.	5
	Conclusion of the experiments evaluated on the pharmacological actions of AR	12
3	Symptoms of estrogen deficiency	18
4	WHO classification for diagnosis of osteoporosis using BMD	
5	Markers of home formed in a 1	22
6	Markers of bone formation and recorption Effect of AR root extract on femoral parameters after 90 days of	23
	treatment	42
7	Effects of AR root extract on biochemical parameters after 90 days of	72
	treatment	47
8	Effects of AR root extract on rats body weight after 90 days of	
0	treatment	59
9	Effects of AR root extract on rats uterine weight after 90 days of treatment	60
10	Effect of AR root extract on thickness of endometrium and myometrium	UU
	after 90 days of treatment	(5

LIST OF FIGURES

Fiş	gures	
	1	The characterictic of Asparagus racemosus Willd
	2	The main active compounds of Asparagus racemosus Willd
		(I)Shatavarin, (II)Sarsasapogenin
	3	Menopausal stages
	4	Diagram of a long bone
	5	Histological section (stained with H&E) of the long bone at
		diaphysis in longitudinal plane of rat showing two types of
		bone tissue: compact and cancellous bone. Scale bars = 200
		μm
	6	Turnover process
	7	The characterictic of Epimedium sagittatum (left figure) and the
		chemical structure of icariin (right figure)
	8	Terpenoid and sterol chemical structure
	9	Daidzin and Genistin chemical structure
	10	The chemical structure of puerarin
	11	Anatomy of human uterus
	12	Anatomy of rat uterus
	13	Histological section (stained with Hematoxylin & Eosin) of the
		uterus of rat in transverse plane. Scale bars = $50 \mu m$
	14	Breeding female rats with an average of 12 mammary glands
	15	Histological features of H&E-stained paraffin section of the
		mammary glands of female Wistar rat showing the epithelium,
		duct, adipose tissue and connective tissue
	16	Experimental schedule

Figures		Page
17	Effect of AR root extract treatment on serum β-CTx measured in various groups of rats. Treatment groups represented are	
	SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE.	
	Bar represent mean \pm S.E.M. of six animals. ap < 0.05 and cp <	
	0.001 compared to SHAM; $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$	
	compared to OVX	44
18	Effect of AR root extract treatment on serum P ₁ NP measured in	
	various groups of rats. Treatment groups represented are	
	SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE.	
	Bar represent mean \pm S.E.M. of six animals. Bar represent	
	mean \pm S.E.M. of six animals. ${}^{b}p$ < 0.01 compared to SHAM;	
	$^{\#\#}p$ < 0.01 compared to OVX	45
19	Histology of the distal femur at metaphyseal region in longitudinal	
	plane in animal that was SHAM operated control and orally	
	administerd with vehicle for 90 days	48
20	Histology of the distal femur at metaphyseal region in longitudinal	
	plane in animal that was ovariectomized and orally	
	administered with vehicle for 90 days after ovariectomy	49
21	Histology of the distal femur at metaphyseal region in longitudinal	
	plane in animal that was ovariectomized and orally adminitered	
	with 100 mg/kg B.W./day of AR root extract for 90 days after	
	ovariectomy	49

1.0

Figures	Ser .	Page
22	Histology of the distal femur at metaphyseal region in longitudinal plane in animal that was ovariectomized and orally adminitered with 1000 mg/kg B.W./day of AR root extract for 90 days after ovariectomy	50
23	Histology of the distal femur at metaphyseal region in longitudinal plane in animal that was ovariectomized and orally administered with 0.1 mg/kg B.W./day of EE for 90 days after ovariectomy	50
24	Effect of AR root extract on the thickness of trabecular bone in various groups of rats. Treatments represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean \pm S.E.M. of six animals. $^{c}p < 0.001$ compared	
25	to SHAM; $^{\#\#}p$ < 0.001 compared to OVX	52
26	groups of rats. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean \pm S.E.M. of six animals. ap < 0.05, bp < 0.01 and cp < 0.001 compared to SHAM; $^\#p$ < 0.05 and $^\#p$ < 0.01 compared to OVX	53
26	Effect of AR root extract on trabecular area in metaphysis of distal femur in various groups of rats. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean \pm S.E.M. of six animals. bp < 0.01 and cp < 0.001 compared to SHAM; $^{\#}p$ < 0.01 and $^{\#\#}p$	
	< 0.001 compared to OVX	54

(1

Figures		Page
27	Cortical bone morphology in longitudinal plane of femur in animal that was SHAM operated control and orally administered with vehicle for 90 days.	55
28	Cortical bone morphology in longitudinal plane of femur in animal that was ovariectomized and orally administered with vehicle	
29	for 90 days after ovariectomy. Cortical bone morphology in longitudinal plane of femur in animal that was ovariectomized and orally administered with 100 mg/kg B.W./day of AR root extract for 90 days after ovariectomy.	55
30	Cortical bone morphology in longitudinal plane of femur in animal that was ovariectomized and orally administered with 1000 mg/kg B.W./day of AR root extract for 90 days after	56
31	ovariectomy Cortical bone morphology in longitudinal plane of femur in animal that was ovriectomized and orally administered with 0.1 mg/kg	56
32	B.W./day of EE for 90 days after ovariectomy Effect of AR root extract on cortical bone thickness in various groups of rats. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent	57
33	mean ± S.E.M. of six animals Uterine morphology in transverse plane in animal that was SHAM operated control and orally administered with vehicle for 90 days. The uterine compartments are epithelium (Ep),	58
	endometrium (E), myometrium (M) and uterine gland (G)	62

0

(1)

Figures		Page
34	Uterine morphology in transverse plane in animal that was ovariectomized and orally administered with vehicle for 90	
	days after ovariectomy. The uterine compartments are	
	epithelium (Ep), endometrium (E), myometrium (M) and	
	uterine gland (G)	63
35	Uterine morphology in transverse plane in animal that was	
	ovariectomized and orally administered with 100 mg/kg	
	B.W./day of AR root extract for 90 days after ovariectomy.	
	The uterine compartments are epithelium (Ep), endometrium	
	(E), myometrium (M) and uterine gland (G)	63
36	Uterine morphology in transverse plane in animal that was	
	ovariectomized and orally administered with 1000 mg/kg	
	B.W./day of AR root extract for 90 days after ovariectomy.	
	The uterine compartments are epithelium (Ep), endometrium	
	(E), myometrium (M) and uterine gland (G)	64
37	Uterine morphology in transverse plane in animal that was	
	ovariectomized and orally administered with 0.1 mg/kg	
	B.W./day of EE for 90 days after ovariectomy. The uterine	
	compartments are epithelium (Ep), endometrium (E),	
	myometrium (M) and uterine gland (G)	64
38	Mammary gland morphology in animal that was SHAM operated	
	control and orally administered with vehicle for 90 days	
	showing the features of duct (D), fibrocollagenous tissue (F)	
	and soft adipose tissue (A)	67

	Figures		Page
	39	Mammary gland morphology in animal that was ovariectomized	
		and orally administered with vehicle for 90 days after	
		ovariectomy showing the features of duct (D),	
		fibrocollagenous tissue (F) and soft adipose tissue (A)	67
ì	40	Mammary gland morphology in animal that was ovariectomized	
		and orally administered with 100 mg/kg B.W./day of AR root	
		extract for 90 days after ovariectomy showing the features of	
		duct (D), fibrocollagenous tissue (F) and soft adipose tissue	
		(A)	68
	41	Mammary gland morphology in animal that was ovariectomized	
		and orally administered with 1000 mg/kg B.W./day of AR	
		root extract for 90 days after ovariectomy showing the	
		features of duct (D), fibrocollagenous tissue (F) and soft	
		adipose tissue (A)	68
	42	Mammary gland morphology in animal that was ovariectomized	
<i>(</i> :		and orally administered with 0.1 mg/kg B.W./day of EE for	
		90 days after ovariectomy showing the features of duct (D),	
		fibrocollagenous tissue (F) and soft adipose tissue (A). Note	
		the minute amount of secretion (S) in the lumen of duct	
		resulting from stimulation of estrogen treatment	69
	43	Effect of AR root extract on the glandular area of mammary gland.	
		Treatment groups represented are SHAM, OVX,	
		OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent	
		mean \pm S.E.M. of six animals. $^{c}p < 0.001$ compared to	
ŀ		SHAM; $^{\#\#}p < 0.001$ compared to OVX	70

ABBREVIATIONS

μm = micrometer

 μm^2 = squaremicrometers

°C = Degree Celcius

A = adipose tissue

ALP = alkaline phosphatase

AR = Asparagus racemosus

 β -CTx = beta-crosslaps

BMD = Bone mineral density

B.W. = Body weight

Ca = Calcium

cm = centrimetre

dL = deciliter

D = duct

DMBA = 7,12-dimethylbenz[α]anthracene

D.W. = Distilled water

E = endometrium

EDTA = Ethylenediaminetetraacetic acid disodium salt dehydrate

EE = 17α -ethynylestradiol

Ep = epithelium

ER = Estrogen receptor

F = Fibrocollagenous tissue

g = gram

1)

G = uteine gland

h = hour

H&E = Hematoxylin and eosin

HRT = Hormone replacement therapy

mg = milligram

min = minute

ml = milliliter

ABBREVIATIONS (CONT.)

mm² = squaremeters

NaOH = Sodium hydroxide

ng = nanogram

Ostex = Aminoterminal cross-linking telopeptide of bone collagen

OVX = Ovariectomy

PG = Propylene glycol

pg = picogram

0

10

9

Pi = Inorganic phosphate

P₁NP = Procollagen type 1 amino-terminal propeptide

SERM = Selective estrogen receptor modulator

SHAM = Sham operate control

S.E.M. = Standard error of the mean

TRAP = Tartrate-resistance acid phosphatase

U = ungstrom