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PHARMACEUTICO-CHEMICAL AND PHARMACOLOGICAL STUDIES ON A CRUDE DRUG FROM *LAGERSTROEMIA SPECIOSA* (L.) PERS.

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ABSTRACT

Various dosage forms were prepared from the crude drug, coming from *Lagerstroemia speciosa* (L.) Pers. or "banaba" and pharmaceutico-chemical and pharmacological studies were done on them. Thin-layer chromatography of all dosage forms showed the presence of B-sitosterol except the fluid-extract prepared using 50% ethyl alcohol. Fluidextracts using 95% and 50% ethyl alcohol produced a diuretic effect in rats while petroleum ether and ethyl ether extracts showed an anti-inflammatory activity in mice.

INTRODUCTION

In the Philippines, one popular home remedy for diarrhea, diabetes and urinary problems is a preparation of tea from the leaves of *Lagerstroemia speciosa* (L.) Pers., locally called "banaba"

Preliminary chemical and pharmacological investigations in 1941 by Dr. Faustino Garcia resulted in the isolation of a hypoglycemic substance which he called an insulin-like principle (Quisumbing, 1951). A chemical investigation of a hypoglycemic preparation, 20% decoction of the leaves, showed that it did not have any of the active compounds commonly found in plants, namely, alkaloids, glycosides, steroids and flavonoids (Carew and Ching, 1961). However, an in-depth study of the plant undertaken at the Tohoku College of Pharmacy showed that the leaves contain lager-acetal, n-amyl alcohol, B-sitosterol and a new tannin called lagertannin (Takahashi et al, 1975).

Considering the earlier studies made and the many anecdotal claims of the medicinal properties of this plant, the authors decided to conduct pharmaceutico-chemical and pharmacological studies to provide the scientific basis for such alleged therapeutic properties and a guide in the preparation of safe and suitable dosage forms. If its medicinal properties are confirmed, this tree which grows easily and abundantly in this country can be utilized as a substitute for imported diuretic, antidiabetic and antidiarrheal drugs. To aid in its identification and quality control when produced commercially, the botanical characteristics of the leaves were also described.

MATERIALS AND METHODS

Plant Material: Leaves were collected at Mt. Makiling, Los Baños, Laguna.

Botanical Studies

Fresh and dried banaba leaves were examined and described morphologically. Slides of histological sections had been prepared and anatomical structures described previously (Castro, 1986). Ground banaba leaves were cleared in chloral hydrate overnight, mounted in chloral hydrate and glycerine solution and examined under the microscope.

Pharmaceutico-Chemical Studies

Analysis of the Crude Drug

The proximate components of the powdered leaves determined are: total ash, acid-insoluble ash, crude fiber, moisture and 6 extractives soluble in alcohol, dilute alcohol, hexane, water and ether (USP XX, 1980).

Preparation of Dosage Forms

Both hot and cold processes were used in preparing the different dosage forms from the powdered leaves.

A. Cold Process

1. *Fluidextract.* Two fluidextracts using 95% (A) and 50% (B) ethyl alcohol as menstruum were prepared by percolation. An alcoholic solution containing the therapeutic constituents of one gram of the drug per mL of the solution was obtained (USP XX, 1980).
2. *Tincture.* This dosage form was prepared by the general process by percolating the powdered drug with ethyl alcohol and obtaining an alcoholic solution of the drug with a strength of 10% by weight (USP XX, 1980).

B. Hot Process.

1. *Decoction.* The powdered drug was boiled in distilled water in a covered container for 15 minutes obtaining an aqueous solution representing 20% w/v of the crude drug (Osol *et al.*, 1975).

2. *Extracts.* The powdered leaves (500 g) were extracted first with petroleum ether (b.p. 35°-60°C), followed with ethyl ether (anhydrous) and finally with ethyl alcohol (95%). Each extract was collected and concentrated in vacuo yielding three crude extracts: petroleum ether, 13.8 g (extract *a*), ethyl ether, 24.5 g (extract *b*), and ethyl alcohol, 55.9 g (extract *c*) (Garcia *et al.*, 1980).

Phytochemical Screening

The suitable dosage forms prepared from the crude drug: decoction (20%), tincture, fluidextracts *A* (95% ethyl alcohol) and *B* (50% ethyl alcohol) and extracts *a* (petroleum ether), *b* (ethyl ether) and *c* (ethyl alcohol) were subjected to general chemical reaction tests to determine the presence of therapeutically active plant constituents.

Thin-layer chromatographic analyses of the three extracts and two fluidextracts with standard B-sitosterol and stigmasterol compounds were also undertaken. Chromatograms were prepared on silica gel G plates and developed in two different solvents: 1) chloroform and 2) chloroform-methanol mixture (95 + 5). The chromatograms were viewed under ultraviolet light before spraying with 50% sulfuric acid (alcoholic) and after drying at 110°C for 10 minutes. The color and R_f values of the spots were determined.

Purification of Crude Extracts

Crude petroleum ether and ethyl ether extracts (extracts *a* and *b*) were partially purified by saponification (Garcia *et al.*, 1985). Extract *a* (30 g) was refluxed for two hours in alcoholic potassium hydroxide solution (240 mL). Upon concentration of the refluxed material the semi-solid residue obtained was dissolved in 300 mL hot water which after cooling was repeatedly extracted with anhydrous ethyl ether. The combined ethereal extracts evaporated to dryness yielded 2.5 g of unsaponified portion. The alkaline solution was then acidified with dilute hydroalcoholic acid, repeatedly extracted with anhydrous ethyl ether and yielded 11.9 saponifiable portion upon evaporation of the solvent. Extract *b* (30 g) was separated into the unsaponifiable, 5.72 g and the saponifiable, 7.7 g portions using the above procedure.

The ethyl alcohol extract (extract *c*), 58 g, was triturated with acetone and the collected solution was evaporated to dryness in vacuo, yielding 25 g of acetone-soluble portion. The residue left after trituration with acetone was collected and dried obtaining 17.5 g designated as the acetone-insoluble portion. Chemical reaction tests of the unsaponifiable and saponifiable portions from extracts *a* and *b* and the acetone-soluble and insoluble portions of extract *a* was undertaken to characterize the components present in the isolates obtained.

Chromatographic Isolation of the Sterol Compound

The strong indication of the presence of sterol and/or terpenoid substances in the petroleum ether extract led to its fractionation on silica gel column. The semi-solid residue (10 g) was packed on a glass column containing 200 g silica gel and eluted with 1 liter of petroleum ether as initial solvent followed with 1 liter mixtures of petroleum

ether with increasing amounts of ethyl ether. One hundred twenty of 100 mL fractions were collected and grouped by thin-layer chromatographic techniques (Garcia *et al.*, 1978).

Combined fraction numbers 41-51 eluted by 25-35% ethyl ether in petroleum ether yielded slightly colored crystalline solids upon concentration in vacuo. Chloroform solution of the crystal gave a green color upon addition of acetic acid anhydride and concentrated sulfuric acid (Liebermann-Burchard test), characteristic of a plant sterol. This compound was repeatedly crystallized in acetone-methanol mixture. Thin-layer chromatography of the compound with authentic samples of B-sitosterol and stigmasterol was undertaken. The silica gel G plates were developed in two different solvent mixtures: a) chloroform and b) chloroform and methanol (95 + 5). The chromatograms were viewed under ultraviolet light using a Chromato-Vue Apparatus before and after spraying with 50% alcoholic sulfuric acid. The infrared spectrum in KBr pellets of the sterol substance was taken on Shimadzu Infrared Spectrophotometer (IR-435). The melting point was determined on a Micro-melting Point Apparatus.

Pharmacological Studies

The different dosage forms prepared from the powdered leaves such as: fluidextracts A and B, extracts a, b and c, decoction and tincture were subjected to the following pharmacological tests on experimental animals.

1. Behavioral Observation Screening Test (Nodine *et al.*, 1941; Malone *et al.*, 1962)

Roughly 3-fold increases in doses of the different dosage forms were injected intraperitoneally to Swiss mice (17-23 g). Changes in awareness, mood, motor activity, CNS excitation, posture, motor incoordination, muscle tone, reflexes and various autonomic signs were observed relative to controls (e.g. untreated mice).

2. Diuretic Activity (Domer, 1971)

Sprague-Dawley rats (150-200 g) which were fasted overnight for 18 hours were given the different dosage forms orally. Each rat was individually caged in the metabolic cage and their urine collected every hour for 5 hours. The percentage of increases in urine volume was computed on both the test drug ("banaba") and positive control (Lasix) based on the negative control (NSS). Comparison of the diuretic effects of the positive control versus the test drug was then undertaken.

3. Anti-inflammatory Activity (Winter *et al.*, 1962)

Rats (150-200 g) in groups of 5 dosed with the various forms of "banaba" were injected with 0.05 mL of 1% carrageenin (in saline solution) into the plantar tissue of the right hind paw of each rat. The foot volumes were then measured by a caliper after 3 hours. Percentage inhibition or protection against edema formation was calculated based on the control.

4. Acute Toxicity Determination

The median lethal dose or LD₅₀ of the different dosage forms were determined on two strains of white mice, the Swiss Albino and the Strong A mice. Five groups of 10 each were used per dosage form. Oral and intraperitoneal administration of the drug were given to the animals and they were observed for drug reactions and toxic signs/death adapting the Modified Hippocratic Observation Method (Malone, 1962). The LD₅₀ was calculated based on the graphical method (Litchfield, 1949) and probit analysis method (Fisher, 1950).

5. Anti-diabetic Activity

Initial fasting blood sugar determination on the test animals (mice and rats) were done using the Nelson-Somogyi method (Nelson-Somogyi, 1944). Blood samples were subsequently collected/extracted at hourly intervals for 4 hours after dosing the animals with the test control drugs and its blood sugar content analyzed.

RESULTS AND DISCUSSION

The crude drug used in this investigation consisted of the dried leaf of *Lagerstroemia speciosa* (L.) Pers. of the Family Lythraceae (Fig. 1).

Botanic characteristics: The leaves are glabrous and coriaceous, the upper surface dark green and the lower surface light green. The lamina is 12-25 cm long and 7-12 cm wide, elliptic to oblong in outline, apex obtuse or shortly acuminate, base acute and margin slightly sinuate. The petiole is usually 1 cm long. Venation is reticulate and prominent lateral veins above the middle of the leaf join each other before reaching the margin.

Histology: The upper epidermal cells of the lamina are polygonal, 4- to 8-sided, with cells as long as wide to twice longer than wide, straight walls and abundant large mucilage cells. The lower epidermal cells are irregularly shaped, 4- to 8-sided, with walls that are slightly sinuous and elongated costal cells enclosing other epidermal cells. The stomata are anomocytic and found in the lower epidermis only. The mesophyll consists of 2 to 3 layers of palisade cells containing large rosette crystals. The midrib contains 3 to 6 layers of collenchyma next to the upper and lower epidermis, a large crescent-shaped bicollateral vascular bundle and smaller vascular bundles above it. The bundle sheath consists of two layers, the inner, sclerenchyma and the outer, parenchyma cells. The ground tissue of parenchyma cells contains large rosette crystals (Castro, 1986).

Powdered banaba leaf: It is light green in color. Present are fragments of epidermis containing large mucilage cells and mesophyll with large rosette crystals. Elongated costal cells of the lower epidermis enclose other epidermal cells containing anomocytic stomata.

Chemical analysis of the powdered drug yielded the following proximate components: moisture, 13.1%; total ash, 5.84%; acid-insoluble ash, 1.13%; diluted alcohol extractive,

15.9%; water-soluble extractive, 15.7%; hexane-soluble extractive, 2.18%; volatile ether-soluble extractive, 2.95%; non-volatile ether-soluble extractive, 0.2%; crude fiber, 19.95%. It is significant to note that foreign organic matter in the crude drug estimated as acid-insoluble ash is within the USP specification of not more than 2%.

Chemical examination of the different dosage forms as shown in Table I indicated the presence of significant amounts of glycosides in fluidextract *B* and extract *c*, tannin in the decoction; fluidextract *A* and *B* and extract *c*; sterol in fluidextract *A* and extract *a*; and organic acid in extract *c* only.

Thin-layer chromatography of the fluidextracts and crude extracts with standard compounds of B-sitosterol and stigmasterol on silica gel G plates with chloroform-methanol solvent mixtures (95 + 5) as developing solvents showed that except for fluidextract *B*, all sample preparations produced one definite spot that has fluorescent color and Rf value similar to that of standard compound B-sitosterol (Fig. 2).

We attempted to purify or isolate the active constituents present in the three extracts of "banaba" leaves as characterized by chemical reaction tests. Extracts *a* (petroleum ether extract) and *b* (ethyl ether extract) with sterol as their main constituent were purified by saponification, while solvent extraction method was applied to isolate the tannin compound in extract *c* (ethyl alcohol extract). Chemical examination of the purified isolates indicated that saponification and solvent extraction failed to separate the sterol and tannin in pure form respectively.

Chromatographic fractionation of extract *a* on silica gel column yielded five isolates. The fourth isolate eluted by 25-35% of ethyl ether in petroleum ether after several crystallization processes in acetone-methanol mixtures produced a white crystalline substance (0.2588g) melting at 130°-135°C. Thin-layer chromatography of this substance on silica gel G plates developed in chloroform-methanol mixture (9 + 1) showed a well-defined single spot with a blue-violet fluorescent color under UV light and Rf value identical with standard compound, B-sitosterol. The infrared spectrum (KBr disks) of this substance (Fig. 3) showed absorption bands identical to that of standard B-sitosterol compound (Fig. 4).

Results of the pharmacological experiments on the different dosage forms of the drug are shown in Tables 2, 3, 4 and 5. In the behavioral observation screening tests, the dosage forms produced depression in the experimental animals as manifested by: 1) decrease in awareness, motor activity, muscle tone and respiratory rate; 2) loss of muscle coordination and piloerection; and 3) frequent urination. The last depressive effect was observed only in extracts *a* and *b*.

To find out if the latter two extracts really produce diuresis in experimental animals, diuretic activity tests were conducted but due to lack of material, only the petroleum ether extract (extract *a*) was tested. From the result of Trial III as shown in Table 3, extract *a* gave a satisfactory increase of urine volume after dosing within the period of 3 hours at a dose of 300 mg/kg. The increase, however, was a little lower than the

positive control drug, Lasix. This result indicates that the petroleum ether extract of "banaba" leaves is a good diuretic.

Results of the anti-inflammatory activity test (carrageenin-induced edema in rat's paw) as shown in Table 4, indicate that extracts *a* and *c* gave a higher percentage protection against edema formation in rats than the positive control, aspirin. Thus, petroleum ether and ethyl alcohol extracts of "banaba" can be used as substitutes for aspirin, an anti-inflammatory agent used in rheumatism and arthritis.

Table 5 contains the median lethal dose (LD₅₀) of each dosage form in the acute toxicity experiments on Swiss mice. It was observed that the tincture is the most toxic, followed by ethyl alcohol, ethyl ether extract and decoction.

Preliminary anti-diabetic activity experiments using "banaba" extracts *a*, *b* and *c* showed no appreciable lowering in blood sugar values as compared with that produced by the positive control drug, insulin. Due to lack of chemicals and equipment, however, further tests were deferred.

SUMMARY

1. Thin-layer chromatography of all dosage forms except fluidextract using 50% ethyl alcohol showed the presence of B-sitosterol.
2. Petroleum ether extract produced a diuretic effect in rats.
3. Petroleum ether and ethyl alcohol extracts were shown to possess anti-inflammatory property.

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Table 1. Chemical examination of different "banaba" dosage forms

Chemical Reaction Tests	Constituents Present	Fluidextracts					Extracts			Tincture	Decoction (20%)
		95% Ethanol (A)		50% Ethanol (B)		Pet. Ether (a)	Ethyl ether (b)	Ethanol (c)			
1. Mayer	alkaloids	no ppt (-)	no ppt (-)	no ppt (-)	no ppt (-)	no ppt (-)	no ppt (-)	no ppt (-)	no ppt (-)	no ppt (-)	no ppt (-)
2. Wagner	alkaloids	no ppt (-)	no ppt (-)	no ppt (-)	no ppt (-)	no ppt (-)	no ppt (-)	no ppt (-)	no ppt (-)	no ppt (-)	no ppt (-)
3. Magnesium + HCL	flavonoids	reddish color (+)	reddish color (+)	reddish color (+)	reddish color (+)	no color (-)	no color (-)	no color (-)	no color (-)	no color (-)	no color (-)
4. Fehling's solution	glycosides	orange ppt (+)	brick red ppt (+)	brick red ppt (+)	brick red ppt (+)	no ppt (-)	no ppt (-)	brick red ppt (+++)	brick red ppt (+)	brick red ppt (+)	brick red ppt (+)
5. Froth	saponins/ organic acids	froth (++)	froth (++)	froth (++)	froth (++)	no froth (-)	no froth (-)	froth (++)	froth (+)	froth (+)	froth (+)
6. Liebermann-Burchard	sterols/ terpenes	blue green color (+++)	blue green color (+)	blue green color (++)	blue green color (++)	blue green color (+++)	blue green color (++)	bluishgreen color (+)	greenish-yellow to green (+)	greenish-yellow to green (+)	greenish-yellow to green (+)
7. Salkowski	sterols/ terpenes	blue green color (+++)	blue green color (+)	blue green color (++)	blue green color (++)	blue green color (+++)	blue green color (++)	reddishblue color (+)	dark green color (+)	dark green color (+)	brownish red color (++)
8. Ferric chloride	tannins	black color (++)	black color (++)	black color (++)	black color (++)	no color (-)	bluish color (+)	black color (++)	blue black color (++)	black color (++)	black color (++)

LEGEND:
 (-) = negative
 (+) = slightly positive
 (++) = moderately positive
 (+++) = strongly positive

Table 2. Behavioral observation screening test of "banaba" dosage forms

Dosage Form	Animal	Solvent	Dose mg/kg	Effect
20% decoction	Strong A mice	Distilled water	5,000	Decreased general activity, exophthalmia, piloerection, hypothermia, straub tail, clonic convulsion followed by death.
			7,500	
			8,750	
			10,000	
			20,000	
Tincture	Swiss mice	10% alcohol	300 1,000	Increased respiration, exophthalmia, straub tail, abnormal posture, staggering gait, loss of righting reflex *
Fluidextract A (95% ethyl alcohol as menstrum)	Strong A mice	Distilled water	500	Decreased general activity, loss of muscle coordination, frequent urination, writhing, loss of pinna reflex, exophthalmia and lowering of body temperature.
			1,000	
Fluidextract B (50% ethyl alcohol as menstrum)	Strong A mice	Distilled water	500	Decreased general activity, increased respiratory rate, writhing, loss of pinna reflex, loss of muscle coordination and exophthalmia.
			1,000	
			2,000	
			4,000	
Extract a (petroleum ether)	Swiss mice	10% Tween 80 in NSS	250	Decreased general activity, excessive grooming, frequent urination, increased sensitivity to hot plate test.
			500	
Extract b (ethyl ether)	Swiss mice	10% Tween 80 in NSS	500	Decreased general activity, slight lowering of body temperature, loss of muscle coordination, increased sensitivity to hot plate test, piloerection and frequent urination, fatal at 2,000 mg/kg and above.
			1,000	
			2,000	
			4,000	
Extract c (ethyl alcohol)	Swiss mice	Distilled water	500	General activity was increased with increased respiratory rate, writhing, loss of pinna reflex and loss of muscle coordination. Exophthalmia for mice at 1,000 mg/kg and above. Fatal at 4,000 mg/kg dose.
			1,000	

Table 3. Diuretic tests on "banaba" dosage forms

Drug	Dose (mg/kg)	% Increase in urine volume				
		1st hour	2nd hour	3rd hour	4th hour	5th hour
Trial I						
Petroleum ether (extract a)	30	-	21.82	24.17	27.16	28.26
	300	-	71.72	60.83	35.80	32.61
Lasix (positive control)	7	412.74	300.00	283.33	191.36	160.81
Trial III						
Petroleum ether extract (extract a)	30	-	200.00	-	200.00	140.00
	300	-	500.00	100.00	125.00	80.00
Lasix (positive control)	7	-	2,100.00	450.00	500.00	500.00
Trial III						
Petroleum ether extract (extract a)	30	-	0	66.66	-	60.00
	300	-	700.00	566.67	380.00	380.00
Lasix (positive control)	7	7,100.00	1,300.00	966.67	540.00	540.00

Table 4. Anti-inflammatory tests on "banaba" dosage forms

Extract/Control drug	Dose (mg/kg)	% Protection against edema
Aspirin	200	11.5
Petroleum ether extract (extract a)	250	23.01
Ethyl alcohol extract (extract c)	250	21.24

Table 5. Acute toxicity tests on "hanaba" dosage forms

Dosage Forms	Solvent	Route of administration	Strain of mice	Dose (g/kg)	Lethal dose (LD ₅₀) (g/kg)
Decoction (20%)	Distilled water	oral	Female Strong A mice	300	375
				350	Lower limit - 300
				400	Upper limit - 468.75
				500	
Tincture	10% Tween 80 in NSS	oral	Female Strong A mice	0.25	0.81
				0.50	Lower limit - 0.61
				0.70	Upper limit - 1.08
				1.0	
Fluidextract A (95% ethyl alcohol as menstruum)	10% Tween 80 in NSS	Intra-peritoneal	Male Strong A mice	0.5	2.634
				1.0	± 10.179
				8.0	
				14.0	
Extract B (50% ethyl alcohol as menstruum)	10% Tween 80 in NSS	Intra-peritoneal	Male Strong A mice	4.0	8.622
				7.0	± 0.482
				8.0	
				14.0	
Extract a (petroleum ether)	10% Tween 80 in NSS	Intra-peritoneal	Male Swiss mice	0.25	not done
				0.5	
				1.0	
Extract b (ethyl ether)	10% Tween 80 in NSS	Intra-	Male Swiss mice	0.5	3.161
				1.0	± 0.170
Extract c (ethyl alcohol)	10% Tween 80 in NSS	Intra-peritoneal	Male Strong A mice	0.75	1.395
				1.0	± 0.95
				1.5	
				2.0	
				3.0	



Fig. 1. Lagerstroemia speciosa (L.) Pers.

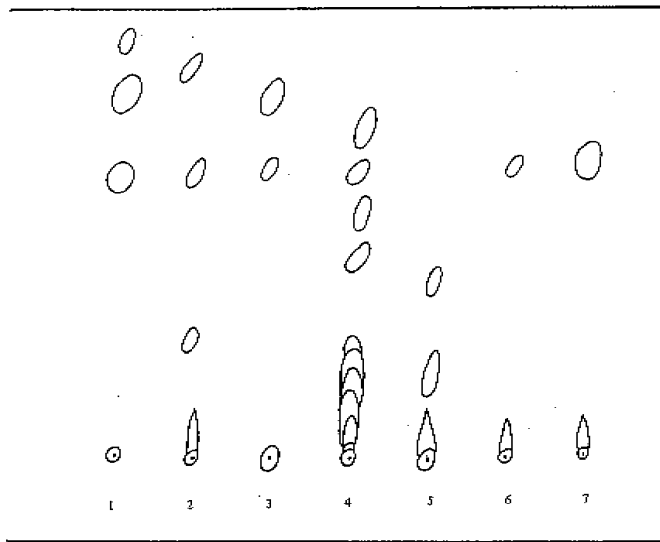


Fig. 2. Thin-layer chromatograms of 1) petroleum ether extract, 2) ethyl ether extract, 3) ethanol extract, 4) fluidextract a, 5) fluidextract b, 6) B-sitosterol and 7) stigmasterol in solvent system chloroform-methanol (95 : 5).



Fig. 3. IR spectrum of "Banaba" sterol (KBr disk)

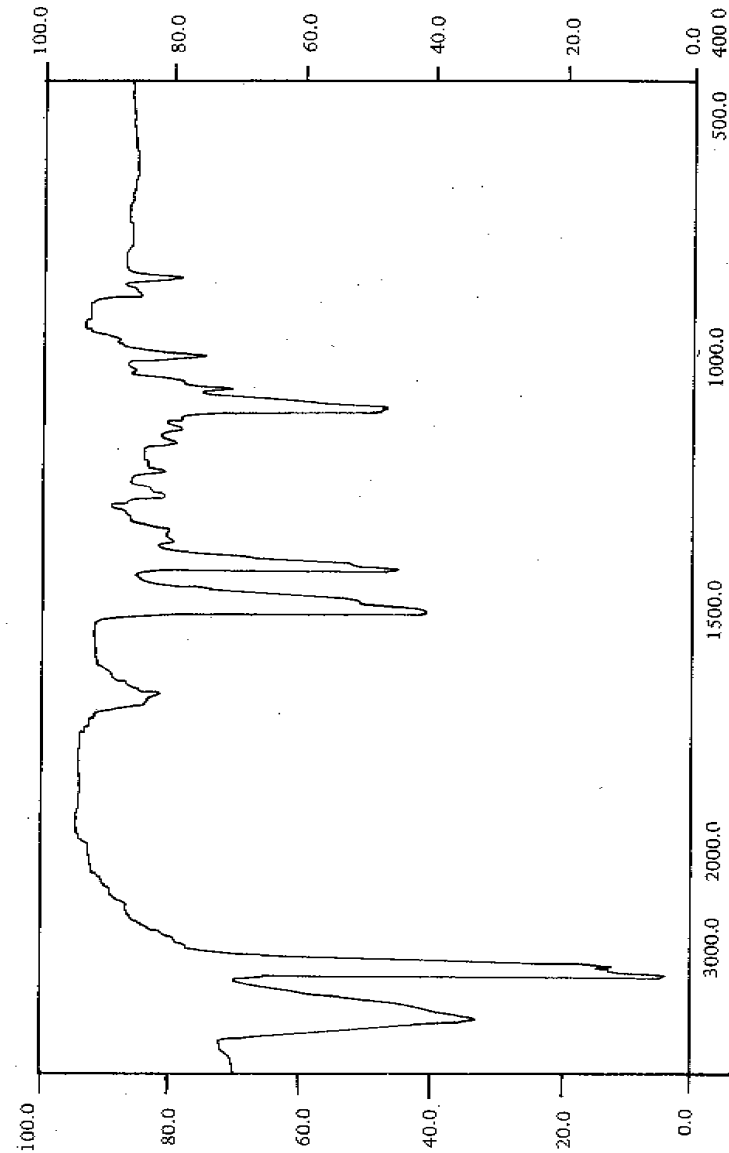


Fig. 4. IR spectrum of standard B-sitosterol (KBr disk)