Do your pets have these kinds of symptoms?

- ☑ Goes to the toilet more often
- ☑ Small amount of urine during urination
- ☑ Blood in the urine
- ☑ Straining while urinating
- ☑ Vocalization during urination
- ☑ Being anxious/restless
- ☑ No urination more than 1 day

If it was on your list…

It could be presence of urolith in the bladder or urinary tract

If the BLADDER and and URINARY TRACT contain UROLITH, what will be happened?

URAJIRON for Urinary Tract Health

A formulation that contain an ingredient that has efficacy for diuresis and body fluid content adjustment.

What is Quercus salicina

Quercus salicina is an evergreen tree of the Fagaceae family that grows naturally in Shikoku, Kyushu of Japan. It has been named "Back White Oak" because the back of its leaves is white with fruity like an acorn.

From ancient times, in Tokushima prefecture, Shikoku Island of Japan, decoction twigs and leaves of dried *Quercus salicina* have been used as materials to prepare a healing tea which helps to excrete bladder stones from the body

Twigs and leaves of *Quercus salicina* contain a lot of tannins. This ingredient promotes the excretion of urine and it can be expected to be useful for adjusting water content in the body.

The main effect of Quercus salicina

Quercus salicina extract increases urine volume by contracting the renal vein. Therefore, is it expected to improve the swelling (edema) by excreting extra fluid in the body. It has also has antibacterial and anti-inflammatory effects which helps to improve the general condition during the course of the disease.

Quercus salicina

Increase the diuresis and body fluid content

Twigs and leaves of ***Querces salicina*** contain a lot of tannins which is beneficial for adjusting body fluid content of the body

Yeast Extract

Natural Amino Acid that confronts the external bacteria and virus.The main ingredients are amino acids, nucleic acid-related substances, minerals, and vitamins, which prevent the invasion of bacteria and viruses as well as support health.

Good Manufacturing Practice (GMP) certificate

It is produced by a domestic pharmaceutical manufacturer. There is domestic pharmaceutical product GMP *Manufactured in a conforming licensed factory.

The producing factory in Japan is licensed with GMP certificate. It is a certification for the safety of medical products which include the manufacturing facility, staff management, quality and productivity management as well as compliance from the workers to the required standard of practice.

It is a 100% plant-based product which can be given to pets with fragile condition safely

It is a highly safe product therefore can be given to pets with fragile condition. It can also be crushed and mix together with wet food if giving tablet is difficult.

Ingredient : *Quercus salicina (sort of oak)* extract powder, powder, yeast extract, pine fiber, crystalline cellulose, lactose, dextrin, sucrose ester

Amount : 60 Tablets

**The standard amount for the body weight 5 kg is 1~2 tablets per day.*

LAPLUS

URAJIROGASHI EXTRACT

For Prevention of Swelling

Urajirogashi (*Quercus salicina*) is a fagaceous evergreen tree which grows wild in Japan. It produces a nut like acorn. Its dried leaves and twigs have been used as a tea for a long time and is experientially known to increase urine and to excrete calculus.

Main component of Urajirogashi extract

Leaves and twigs of Urajirogashi have much tannins, which promote urinary excretion by stimulation of ureters.

Diuretic function of Urajirogashi extract

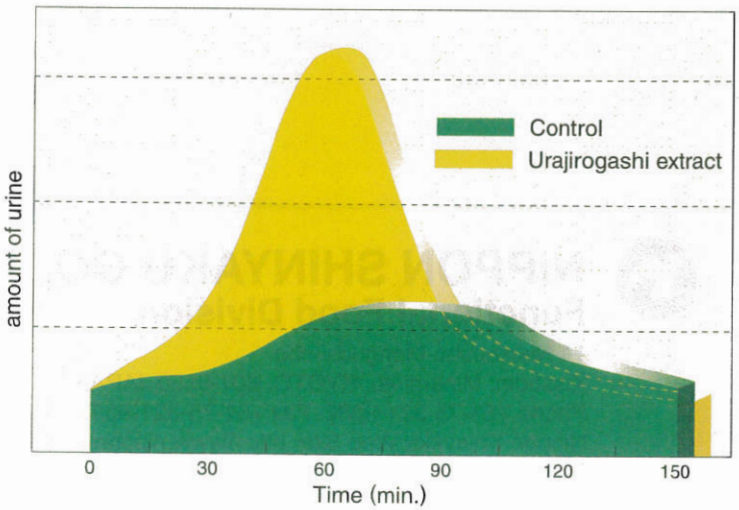
Dr.Oguni has reported that Urajirogashi extract has an effect on urinary output due to contraction of a kidney blood vessel.[1] Dr.Ohsumi has suggested that Urajirogashi extract may help maintain a healthy urinary tract by it's anti-inflammation activity.[2]

Possible applications of Urajirogashi extract are;

1) To reduce swelling in the body
2) To improve urinary excretion
3) To reduce the high blood pressure
4) To lower the value of uric acid
5) To promote weight loss
6) To dissolve calculus in the urinary tract

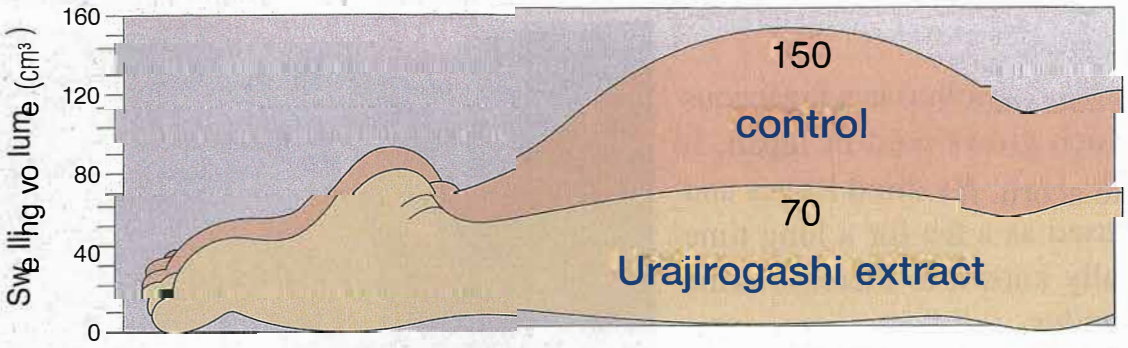
Effect of Urajirogashi extract on humans

Transient and sharp diuretic effect has been confirmed in human studies.



Prevention of Swelling

Urajirogashi extract decreases swelling of calves during daily life.



Safety of Urajirogashi extract

Urajirogashi has been consumed for a long time in Japan, so it is very safe. And in animal acute,sub-acute and chronic toxicity studies tests (in mice and rats), no side effects have been seen.

(References)

1) *Masao Oguni, Shikoku Medical Magazine, 14(90), 602(1959)*

2) *Yoshitugu Ohsumi, Modern Clinicals, 1(9), 598(1967)*

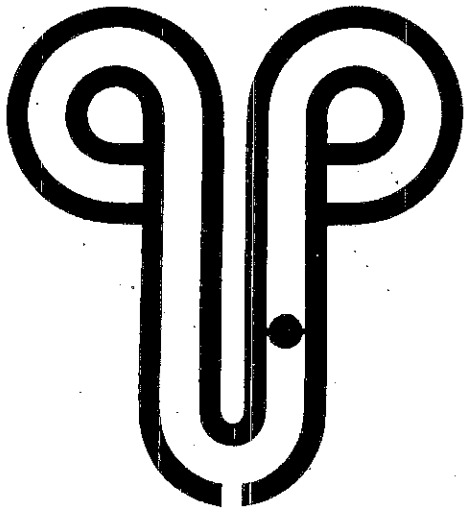
PETS CORNER SINCE 1978

Manufactured for & Distributed By:
Pets Corner Sdn Bhd.
Lot 30, Jalan 4/32A, Pusat Perusahaan Kepong,
Batu 6 1/2, Jalan Kepong, 52100 Kuala Lumpur, Malaysia Tel :+603-6259 7381
& +603-6259 7387
Website : www.petscorner.com.my

www.petscorner.com.my
enquiry@petscorner.com.my
Customer Careline: 019-290 5157 / 019-224 0422
customercare@petscorner.com.my

Oral Remedy for Urinary Calculus

Urocalun



NIPPON SHINYAKU CO., LTD.

Oral Remedy for Urinary Calculus

Urocalun

In Japan a number of plants have been used as medicinals from long ago, and among them is "Urajiro-gashi", a tall tree belonging to Fagaceae.

Recently a series of pharmacological studies have been made on the extract of this tree and as the result a calculus-dissolving or calculus formation-inhibiting action in experimental urinary calculus, anti-inflammation action and diuretic action have been observed in it. Upon clinical application distinct discharging effect on the calculus in upper urinary tract has been established and thus it has been introduced as a novel therapeutic for urinary calculus.

On "Urajiro-gashi":

Urajiro-gashi has the academic name of Quercus salicina Blume or Quercus stenophylla Makino and is an ever-green, great tall tree that belongs to Fagaceae.

Its native place is central to southern regions of Japan and Southern Korea and it stands by nature or by cultivation on a slope of mountain in warm or subtropical area with adequate rainfall.

Studies up to the present time on the constituents of Urajiro-gashi have shown that the extract contains as components pyrogallol, gallic acid, ellagic acid, D-catechin, quercetin and other substances which were separated and identified. But it has now been known that a considerable number of other unknown substances are also contained and future studies are required.

Components and composition:

Urocalun is a capsulated drug, green in color, and each capsule contains 225 mg of the extract of Urajiro-gashi.

Characteristics:

(1) In controlled clinical trial by double blind method, it has been established that Urocalun increases the discharging rate of the calculus in upper urinary tract and also shortens the time required for the calculus discharge.

(2) Urocalun also has long lasting calculus growth-inhibiting action and pain-relieving action, and accordingly it may be administered as a therapeutic with wide range of actions.

(3) It shows few ill side effects and so it may be administered continuously for a long period if required.

Pharmacological Actions:

(1) Calculus formation-inhibiting action

Calculus with known weight, which had been formed by silk thread nucleus method (component being calcium phosphate and magnesium phosphate), was inserted into bladder of a rat, and after oral administration of the extract of Urajiro-gashi (abbridged as Q.S.E. hereafter) in a daily dose of 1,000 mg per head for 60 days the animals were operated to isolate the calculus for weighing and comparing the state of growth of the calculus with the control. The result is shown in Figure 1.

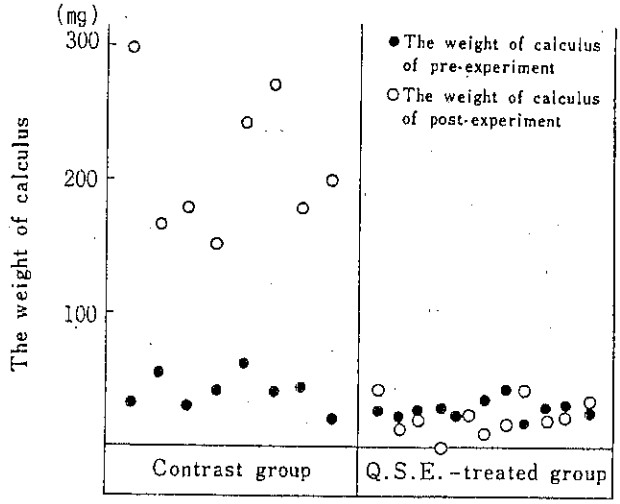


Fig. 1 Formative supperssing action against experimental bladder stone of Q.S.E.

It is clear that in the Q.S.E.-treated group the calculus lost the weight by average 4.22 mg revealing a prominent calculus formation-inhibiting action of the extract. In some cases the calculus was eliminated completely while in the other weakening and dissolving of the calculus were also observed. By contrast, the weight of calculus was increased in the control, the mean gain being 170 mg.

(2) Anti-edema action

On the experimental induced edema by formalin, croton oil, serotonin, egg albumin, and dextran in rat, Q.S.E. showed suppressing action and the effect was most evident on the edema caused by serotonin.

In the experiments for comparison with other anti-inflammatory agents on dextran edema, oral administration of 2,000 mg of Q.S.E. per kg revealed more powerful action than aminopyrine, as shown in Figure 2, and from the therapeutic index Q.S.E. is considered to have an anti-edema action comparable to aminopyrine.

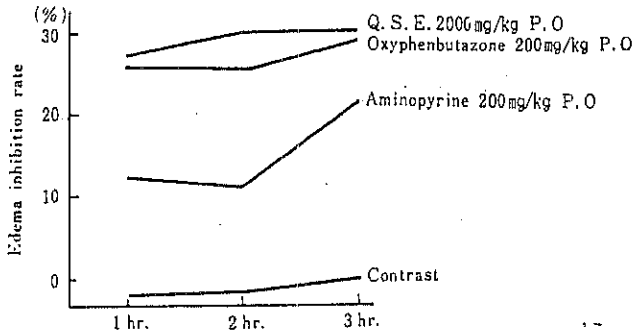


Fig. 2 Suppressing effect against Dextran edema of Q.S.E.

(3) Vascular permeability-suppressing action

Q.S.E. showed a suppressing action on the augmentation of vascular permeability caused by histamine, seronin, and carrageenin in rabbit, and like its anti-edema action, the suppressing action on the permeability augmentation was most powerful on the latter caused by serotonin. As shown in Table 1 Q.S.E. distinctly reduces the area and amount of permeated Evans blue.

Table 1. Vascular permeability-suppressing effect of Q.S.E. (Phlogistica Serotonin)

	Evans blue The area of exudate(mm^2)	Evans blue The volume of exudate(mg/one spot)
Contrast	69.1 ± 8.2	145 ± 13
Q.S.E1000mg/kg	41.0 ± 11.4	106 ± 20

(4) Pleurisy-inhibiting action

Q.S.E. revealed an inhibiting action on experimental pleurisy caused in rat by injection of Evans blue solution and reduced the volume of exudate and the amount of protein in it, particularly the latter. Figure 3 shows that oral administration of 2,000 mg Q.S.E. per kg has an inhibiting action more powerful than the control drug, aminopyrine (200 mg/kg).

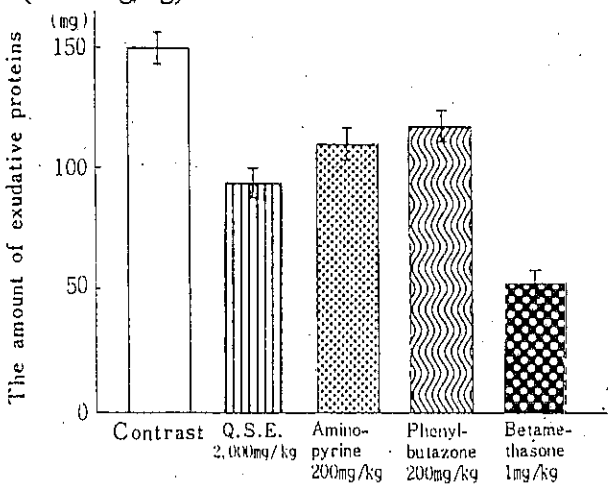


Fig. 3 Q.S.E. suppressive effect on exudative protein in pleural fluid

(5) Diuretic action (Clinical pharmacological study)

In patients with urinary calculus but no extensive kidney function disturbances, 500 ml of water was allowed to take in and urinary excretion after intervals was measured in comparison between the groups with (2 capsules of Urocalun) and without Q.S.E. The results are shown in Figure 4. When Q.S.E. was given maximum urination took place after 60 minutes and the amount of urine was 65 ml/10 minutes whereas in the control maximum urination took place after 90 minutes and the amount of urine was 25 mg/10 minutes only. Therefore the temporary diuretic action of Q.S.E. was proved.

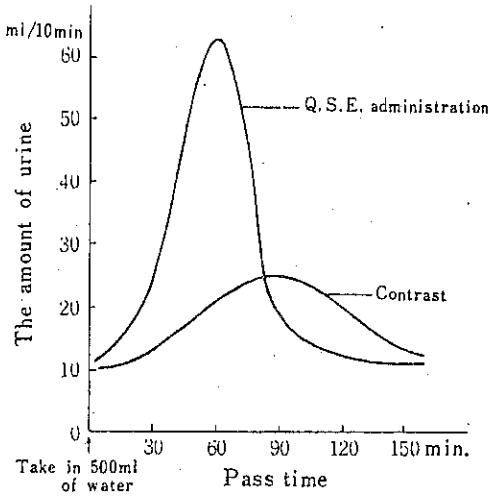


Fig. 4 Diuretic action of Q.S.E.

(6) Toxicity

Acute toxicity: The acute toxicities examined in rat by different methods of administration were as shown in Table 2. In any type of administration the toxicity was extremely low.

Table 2. Acute toxicity

Animal	Administration	LD_{50}	
		Male	Female
Rat	Intraperitoneal	370mg/kg	400mg/kg
	Hypodermic	21,000mg/kg	18,500mg/kg
	Oral	23,000mg/kg over	23,000mg/kg over

Chronic toxicity: After oral administration of Q.S.E. in rat continuously for 180 days in rather large dose, no abnormal findings were observed due to the administration in general symptoms, and in anatomical and pathohistological examinations of main organs.

Clinical results:

Urocalun has been clinically studied in some dozen clinical divisions of universities and great hospitals over the country.

The clinical trials have involved 294 patients with calculi in upper urinary tract (191 patients of ureteral calculi and 103 of kidney culculi).

The clinical effects obtained by Urocalun are thought due to the following properties of the drug:

a) Inhibitory effects on the growth of calculi.

b) Anti-edema and anti-inflammatory actions.
These actions heal the chronic inflammation at the impacted site of calculi to dissociate them.

c) Accelerative effects on ureteral peristalsis by its diuretic action.

(1) Cases of ureteral calculi

As summarized in Table 3, of the 191 cases of ureteral calculi, passage of the calculi was found in 125 cases (65%) and the decending of the calculi in 15 cases (8%).

The effective rate classified by calculus size (maximum diameter in X-ray photograph) was as follows; of 36 cases with small calculi (less than 5 mm in diameter), passage of the calculi was found in 31 cases, and decending of the calculi in 1 case (88.9%); of 135 cases with middle sized calculi (more than 5 mm and less than 10 mm), passage in 84 and decending in 11 (70.4%); the results indicate the remarkable effects of Urocalun. Moreover it is noteworthy that, of 20 cases with large calculi (more than 10 mm), in 10 cases passage was found and in 3 cases decending (65%).

Table 3. Effective ratio of Urocalun on urinary calculus.

	Size of calculus			Total
	Small	Middle	Large	
Case	36	135	20	191
Passage(%)	31(86.1)	84(62.2)	10(50.0)	125(65.4)
Decending(%)	1(2.8)	11(8.2)	3(15.0)	15(7.9)
Total effective ratio(%)	88.9	70.4	65.0	73.3

In passage cases, days required for discharging calculi were less than 14 days in 61.2%, and less than 21 days in 80.6% of the cases with small calculi; less than 14 days in 45.9%, and less than 21 days in 63.5% of middle cases; less than 14 days in 40% and less than 21 days in 60% of the large cases.

(2) Cases of kidney calculi

In 17 cases of 103, Urocalun showed passage or decending of calculi. Bisides, in the patients whose calculi had not moved, Urocalun inhibited their growth for a long time.

(3) Effect on incrustation of indwelling catheter

It was reported that Urocalun prevented the catheter incrustation.

The results of the controlled study by double blind method are as follows.

Though the above clinical results prove the effectiveness of Urocalun on calculi passage in full, controlled studies were carried out in double blind method to avoid mistaking spontaneous stone passage for drug actions; multiclinically in the three institutions of our group (Kyoto University, Nagoya City University, and Mie Prefectural University).

Table 4 shows the total results obtained in those three universities. Within 5 weeks the passage of urinary stone was observed in 32 cases of 52 (61.5%) in the Urocalun administrated group, but only in 23 cases of 51 (45.1%) in the placebo group.

The statistical analysis of the above results shows that Urocalun is significantly effective comparing with placebo. The average required period for stone passage was 13.8 days in the Urocalun group, while it was 18.0 days in the placebo group. That is, Urocalun shortens passage periods more remarkably than placebo.

Table 4. Comparison of Urocalun with placebo on urinary calculus.

Drug	Urocalun	Placebo
Case	52	51
Passage(%)	32(61.5)	23(45.1)
Average days required for stone passage	13.8	18.0

Side effects:

Urocalun has a low toxicity and a excellent tolerance. It rarely causes slight gastrointestinal disorder (discomfort at stomach, diarrhoea etc.), but is in no case so severe that the administration must be discontinued.

Dosage and administration:

Usually 2 capsules, 3 times a day.
Dosage should be individualized according to age and condition.

Indications:

Accerelation of stone passage in ureteral calculi or nephritic calculi.

Package:

100 Cap., 500 Cap.

Isolation of inhibitory substance acting on angiotensin converting enzyme from the leaf of *Quercus stenophylla* MAKINO

Kenji KAMEDA,[a] Takeshi TAKAKU,[a] Hiromichi OKUDA,*[b] Yoshiyuki KIMURA,[b] Takuo OKUDA[c] and Shigeru ARICHI[d]

[a] *Central Research Laboratory, School of Medicine, Ehime University*
[b] *2nd Department of Medical Biochemistry, School of Medicine, Ehime University*
[c] *Faculty of Pharmaceutical Sciences, Okayama University*
[d] *The Research Institute of Oriental Medicine, Kinki University*

(Received January 5, 1987. Accepted April 22, 1987.)

Abstract

The leaf of *Quercus stenophylla* MAKINO (*Fagaceae*) has been traditionally used for treatments of urolithic and hypertensive diseases in Japan, especially in Tokushima Prefecture. We studied the inhibitory effects of the various fractions extracted from the leaf of *Q. stenophylla* on angiotensin converting enzyme activity. Since the methanol extract was shown to exhibit an inhibitory effect on angiotensin converting enzyme, the active substance was further fractionated by silica gel column chromatography after treatment with chloroform. The crystallized active principle was identified as (+)-catechin. Not only authentic (+)-catechin but also its stero-isomer, (−)-epicatechin were shown to be the strong inhibitor of angiotensin converting enzyme and they act as competitive inhibitors for the enzyme.

Key words ACE activity, (+)-catechin, *Quercus stenophylla* MAKINO
Abbreviation ACE, angiotensin converting enzyme

Introduction

The leaf of *Quercus stenophylla* MAKINO (*Fagaceae*) (Japanese name : Urajirogashi) has been used for treatment of urolithic and hypertensive diseases.

Recently, Nishioka *et al.* isolated various tannins such as ellagitannins containing a salidroside (*p*-hydroxyphenethyl alcohol 1-*O*-*β*-D-glucoside) and proto-quercitol gallates *etc.* from the bark of *Q. stenophylla* MAKINO.[1-7] However, biological activities of these tannins are remained to be elucidated. The present experiments were designed to clarify biological activities of the tannins and their related compounds, especially focusing on hypertension.

Renin is well known to be released in blood stream from kidney and converted angiotensinogen to angiotensin I, which is then hydrolyzed to angiotensin II by angiotensin converting enzyme (ACE).[8] It is well known that angiotensin II has hypertensive activity. Recently, some inhibitors of *in vitro* ACE activity such as captopril (SQ 14,225) and nonapeptide (SQ 20,881) have been widely used for the treatment of hypertension.[9,10]

In this study, we attempted the isolation of substances inhibiting ACE activity from the leaf of *Q. stenophylla*.

Materials and Methods

Materials : A Hippuryl-L-Histidyl-L-Leucine (Hip-His-Leu) was obtained from Sigma Co. and used as a substrate for angiotensin converting enzyme (ACE). 2.5 mM Hip-His-Leu was dissolved in 100 mM phosphate buffer containing 300

*〒 791-02 愛媛県温泉郡重信町志津川
愛媛大学医学部第二医化学教室 奥田拓道
Shigenobu-cho, Onsen-gun, Ehime 791-02, Japan

Journal of Medical and Pharmaceutical Society for WAKAN-YAKU 4, 43－48, 1987

mM NaCl (PBS, pH 8.3). Test compounds were dissolved in PBS (pH 8.3). Column chromtography was carried out using silica gel 60 (Wako Chemical Co.) as the absorbent. Other chemicals were reagent grade.

Preparations of angiotensin converting enzyme (ACE) : ACE were isolated from the rat lung by the methods of Takada *et al.*[11] ACE were dissolved in PBS (pH 8.3).

Measurement of inhibitory effects on ACE activity : A mixture of 2.5 mM Hip-His-Leu (0.15 ml) and ACE solution (0.1 ml ; ACE activity : 2.64 units/mg protein, 1 unit : 1 μmoles/hippuric acid/min/ml reaction mixture) were incubated with or without indicated amounts of test compounds at 37℃ for 30 min in a final volume of 0.35 ml. The reaction was stopped by adding 1 N HCl (0.25 ml) and the mixture was extracted with ethyl acetate (2.0 ml). The ethyl acetate phase (1.0 ml) was evaporated, and the residue was dissolved in water (2.0 ml). And then, the free hippuric acid was determined by ultraviolet (UV) absorption at 280 nm for detection.

Isolation of the inhibitory substances from the leaf of Q. stenophylla on ACE activity : The leaf (500 g) of *Q. stenophylla* MAKINO collected in Tokushima Prefecture of Japan was extracted with methanol (1 l×2) at 37℃ for 3 hrs, and then the methanol solutions were concentrated *in vacuo* to give the methanol extracts. The methanol extracts were suspended in water, the suspensions were extracted with chloroform and divided into two fractions, chloroform-insoluble and chloroform-soluble fractions, respectively. The chloroform-insoluble fraction was chromatographed on a silica gel column with acetone-methanol-water (6 : 2 : 1, v/v) and further rechromatographed on a silica gel column to fractionate an inhibiotry substance.

Results

Isolation of ACE inhibitory substances from the leaf of Quercus stenophylla

The leaf of *Q. stenophylla* was extracted with methanol and the resulting extract was concentrated *in vacuo*. The residue was then suspended in water and extracted with chloroform, divided into two fractions, chloroform-insoluble and chloroform-soluble fractions. The preparation procedure was outlined in Fig. 1.

As shown in Fig. 2, both the methanol extracts and chloroform-insoluble fractions were found to inhibit the ACE activity.

Furthermore, the chloroform insoluble fraction was chromatographed on a silica gel column with acetone-methanol-water (6 : 2 : 1, v/v) as the eluants. The active fraction was rechromatographed on a slica gel column to give an inhibitory substance on ACE activity (Fig. 3). The inhibitory substance was obtained as a colorless crystalline powder having mp. 171—174 and $(\alpha)D^{20}$ +17.1 (c=1.01, ethanol) by recrystallization from a mixture of methanol and chloroform. The infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra of the inhibitory sub-

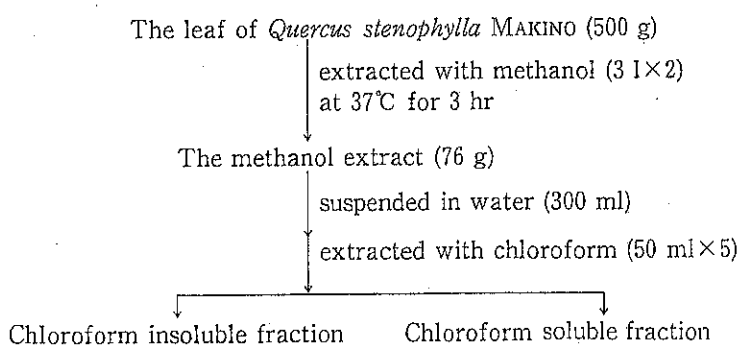


Fig. 1 The preparations of various fractions of the leaf of *Q. stenophylla*.

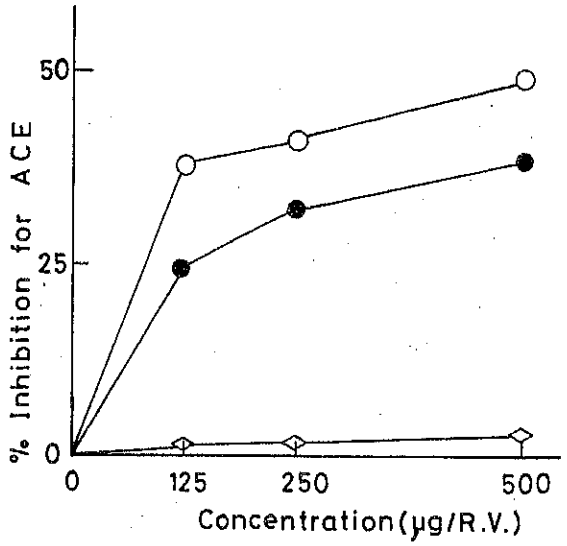


Fig. 2 Inhibitory effects of the various fractions of the leaf of *Q. stenophylla* on ACE activity.
Values are means for 2 experiments. •, methanol extracts ; ○, chloroform insoluble fraction ; ◇, chloroform soluble fraction ; R.V., reaction volume.

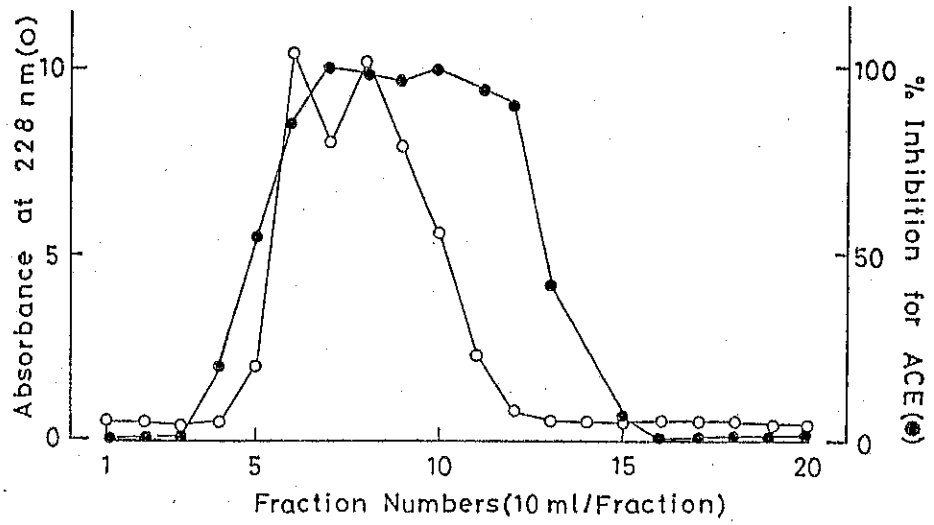


Fig. 3 Isolation of inhibitory substances from the leaf of *Q. stenophylla*.
The chloroform insoluble extracts (2 g) were chromatographed on a silica gel column (ϕ 1.5 cm×80 cm) with acetone-methanol-water (6 : 2 : 1) as the eluant.

stance are identical with those of authentic sample of (+)-catechin. Furthermore, the melting point was not depressed on admixture with the authentic sample of (+)-catechin. And the inhibitory substance was identified as (+)-catechin. (+)-Catechin showed of the dose-dependent inhibition for ACE activity (Fig. 4).

Effects of (+)-catechin and (−)-epicatechin on ACE activity

As the inhibitory substance isolated from the leaf of *Q. stenophylla* on ACE activity was identical with (+)-catechin, (−)-epicatechin, a stereoisomer of (+)-catechin was subjected to examination of an inhibitory action on ACE activity. It

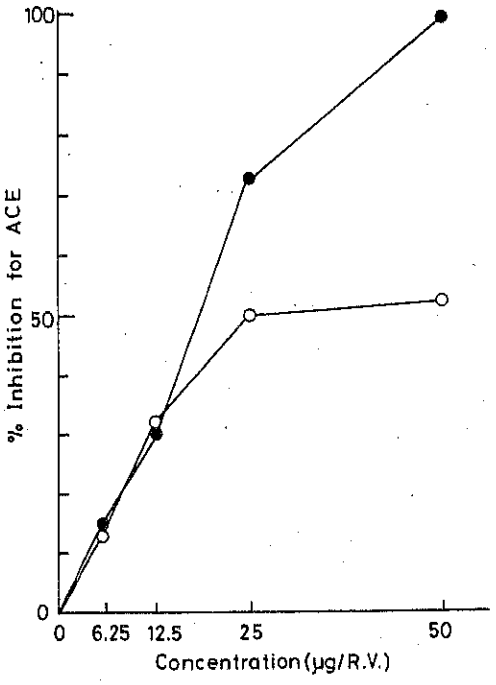


Fig. 4 Inhibitory effects of (+)-catechin and (−)-epicatechin on ACE activity.
Values are means for 2 experiments.
○, (+)-catechin ; ●, (−)-epicatechin ; R.V., reaction volume.

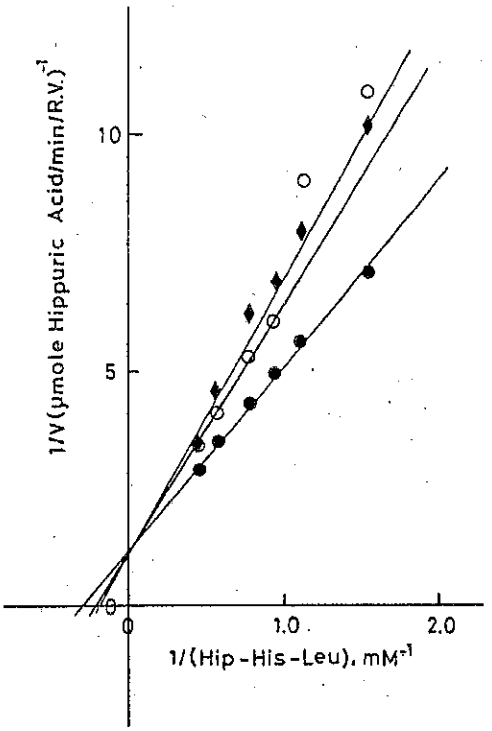


Fig. 5 Lineweaver-Burk plot of ACE with Hip-His-Leu as substrate in the presence or absence of (+)-catechin and (−)-epicatechin.
●, Hip-His-Leu alone ; ○, (+)-catechin (15 μg/reaction volume) ; ◆, (−)-epicatechin (15 μg/reaction volume).

was found that (−)-epicatechin also strongly inhibited the ACE activity. For characterization of the mechanism of inhibition of ACE by (+)-catechin and (−)-epicatechin, the enzyme activity was assayed at various concentrations of Hippuryl-L-Histidyl-L-Leucine (His-His-Leu) as a substrate in the presence and absence of (+)-catechin or (−)-epicatechin. A Lineweaver-Burk plot of the data in Fig. 5 shows that (+)-catechin and (−)- epicatechin inhibited ACE activity competitively. The V_{max} value of the ACE for Hip-His-Leu (substrate) was 8.1×10^{-4}M. The 50% inhibitory concentration (IC_{50}) values of (+)-catechin and (-)-epicatechin for ACE activity were 1.55×10^{-4}M and 2.16×10^{-4}M, respectively.

Discussion

The renin-angiotensin system is one of the major homeostatic mechanisms regulating arterial pressure and salt and water balance.[12,13] The renal enzyme renin, reacting with a substrate present in blood, forms firstly an inactive decapeptide, angiotensin I ; angiotentin I is then converted to the active octapeptide, angiotensin II by angiotensin converting enzyme (ACE).[8] It is well known that angiotensin II has a hypertensive activity. It has been reported that the nonapeptide SQ 20,881, Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro is shown to be a specific and potent inhibitor of angiotensin converting enzyme *in vitro* and *in vivo*.[10] Ondetti *et al.*[10] found that angiotensin converting enzyme, like carboxypeptidase A, was a zinc-containing metalloprotein. Moreover, they suggested that the active site of angiotensin converting enzyme would be similar to that carboxypeptidase A. And they hypothesized that the active site of this enzyme might have a group

capable of interacting with the COOH-terminal amide bond of the substrate, probably through hydrogen bonding, and the zinc ion must be suitably located at the active site of this enzyme to polarize the carbonyl groups of the scissile amide bonds making it more sussceptible to hydrolytic cleavage. For the mechanisms of an angiotensin converting enzyme inhibitor, SQ 14,225 (captopril), Ondetti *et al*.[10] suggested that the interaction of the carboxyl group with the zinc atom of this enzyme plays an important role in determining the inhibitory potency.

Okuda *et al*.[14] reported that three tannins such as geraniin, punicalin and punicalagin, and a related compound (−)-epigallocatechin gallate interacted with the heavy metal ions such as Fe^{3+}, Cu^{2+}, Pb^{2+}, Cr^{6+} and Hg^{2+}. The present studies were found that (+)-catechin isolated from the leaf of *Q. stenophylla* and (−)-epicatechin were competitive inhibitor for angiotensin converting enzyme activity. These results suggest that (+)-catechin and (−)-epicatechin might be interacted with the zinc atom in angiotensin converting enzyme, and they might cause the competitive inhibitions for angiotensin converting enzyme in cosequence. Furthermore, it is found that the inhibitory effects of (+)-catechin on angiotensin converting enzyme activity are stronger than those of (−)-epicatechin. These results suggest that the difference of inhibitory actions of (+)-catechin and (−)-epicatechin on angiotensin converting enzyme may be due to stero-configuration of these compounds.

It was thus of great interest that (+)-catechin was firstly isolated from the leaf of *Q. stenophylla* or medicinal plants as the inhibitory substances on angiotensin converting enzyme, and flavan-3-ol such as (+)-catechin and (−)-epicatechin were structurally different from the well-known angiotensin converting enzyme inhibitor, such as, nonapeptide SQ 20,881 and captopril SQ 14,225.

Experiments are further needed to examine their clinical significances in the treatment of hypertension.

和文抄録

ウラジロガシ葉 (*Quercus stenophylla*) は日本，特に徳島県下で尿路結石および高血圧の治療薬として民間的に用いられている。著者らはウロジロガシ葉の抽出物を分画し，その各フラクションのアンギオテンシン変換酵素に対する阻害効果を検討した。その結果，メタノール抽出エキスがアンギオテンシン変換酵素活性を阻害することが判明したので，その活性成分はメタノール抽出エキスをクロロホルムで処理した後，シリカゲルカラムクロマトグラフィーによって分離した。その阻害物質は (+)-catechin として同定した。(+)-catechin ばかりでなく立体異性体である (−)-epicatechin もまた強い阻害効果を示し，そして，両者はアンギオテンシン変換酵素に対して拮抗阻害剤として作用した。

References

1) Nonaka, G., Nishimura, H. and Nishioka, I.: Tannins and related compounds. IV. Seven new phenol glucoside gallates from *Quercus stenophylla* MAKINO. (1). *Chem. Pharm. Bull.* **30**, 2061-2067, 1982

2) Nishimura, H., Nonaka, G. and Nishioka, I.: Tannins and related compounds. XVIII: A gallotannin and two ellagitannins containing a *p*-hydroxyphenethyl alcohol 1-*O*-*β*-D-glucoside (salidroside) core from *Quercus stenophylla* MAKINO. (2). *Chem. Pharm. Bull.* **32**, 1735-1740, 1984

3) Nishimura, H., Nonaka, G. and Nishioka, I.: Tannins and related compounds. XIX. Eight new gallotannins containing a protoquercitol core from *Quercus stenophylla* MAKINO. (3). *Chem. Pharm. Bull.* **32**, 1741-1749, 1984

4) Nishimura, H., Nonaka, G. and Nishioka, I.: Tannins and related compounds XX. Two new ellagitannins containing a protoquercitol core from *Quercus stenophylla* MAKINO. (4). *Chem. Pharm. Bull.* **32**, 1750-1753, 1984

5) Nishimura, H., Nonaka, G. and Nishioka, I.: Seven quinic acid gallates from *Quercus stenophylla*. *Phytochemistry* **23**, 2621-2623, 1984

6) Nonaka, G., Nishimura, H. and Nishioka, I.: Tannins and related compounds. Part 26. Isolation and structures of Stenophyllanins A, B, and C, novel tannins from *Quercus stenophylla*. *J. Chem. Soc. Perkin Trans.* I, 163-172, 1985

7) Nishimura, H., Nonaka, G. and Nishioka, I.: Tannins and related compounds. XLVI. Isolation and structures of stenophynins A and B, novel tannins from *Quercus stenophylla* MAKINO. *Chem. Pharm. Bull.* **34**, 3223-3227,

1986

8) Skeggs, L. T., Marsh, W. H., Kahn, J. R. and Shumway, N. P. : The existence of two forms of hypertension. *J. Exp. Med.* **99**, 275-282, 1954

9) Atkinson, A.B. and Robertson, J.I.S. : Captopril in the treatment of clinical hypertension and cardiac failure. *Lancet* **II**, 836-839, 1979

10) Ondetti, M.A., Rubin, B. and Cushma, N.D.W. : Design of specific inhibitors of angiotensin-converting enzyme : New class of orally active antihypertensive agents. *Science* **196**, 441-444, 1977

11) Takada, Y., Uno, M., Hiwada, K. and Kokobu, T. : Biochemical and immunological studies of angiotensin converting enzymes from human, bovine, dog, hog, rabbit, rat and sheep kidneys. *Comp. Biochem. Physiol.* **73B**, 189-194, 1982

12) Brown, J.J., Fraser, R., Leckie, B., Lever, A.F., Morton, J.J., Padfield, P.L., Semple, P.F. and Robertson, J.I.S. : Significance of renin and angiotensin in hypertension. *Cardiovasc. Clin.* **9**, 55-89, 1978

13) Haber, E. : The role of renin in normal and pathological cardiovascular homeostasis. *Circulation* **54**, 849-861, 1976

14) Okuda, T., Mori, K., Shiota, M. and Ida, K. : Effect of the interaction of tannins with coexisting substances. II. Reduction of heavy metal ions and solubilization of precipitates. *Yakugaku Zasshi* **102**, 735-742, 1982

Biosci. Biotech. Biochem., 56 (9), 1478—1479, 1992

Note

Inhibitory Effect on Lipase Activity of Extracts from Medicinal Herbs

Susumu SHIMURA, Wakako TSUZUKI,* Shoichi KOBAYASHI,* and Tateo SUZUKI*

Lotte Central Laboratory Co., Ltd., Numakage, Urawa 336, Japan
**National Food Research Institute, Ministry of Agriculture, Forestry, and Fisheries, Kannondai, Tsukuba 305, Japan*
Received February 18, 1992

Lipase inhibitors can be usd to prevent undesirable changes in foods such as an off-flavor, which may be caused by lipase, and also in pharmaceuticals. It has been reported that natural materials such as phosphatidyl choline,[1] the proteins in soybean[2,3] and various cereals,[4] and tannin[5] showed inhibitory activity toward lipase. It has been demonstrated that the inhibition by phosphatidyl choline and proteins was caused by the indirect interaction between the inhibitor and lipase.[1,6] However, few lipase inhibitors have been detected in natural materials. This paper describes the screening of lipase inhibitors from the water-soluble components of medicinal herbs which display various pharmaceutical actions.

The extract from medicinal herbs was prepared from 0.4 g of a powdered sample, which was homogenized with 10 ml of McIlvaine buffer (0.1 M citric acid–Na_2HPO_4, pH 7.4), and filtered after extracting for 1 day at room temperature. Lipase activity was determined by the method of Jacks *et al.*[7] with a fluorescent substrate. A 0.05 ml aliquot of porcine pancreatic lipase (Sigma type II, 0.085 mg/ml of McIlvaine buffer (pH 7.4)) and 0.05 ml of McIlvaine buffer were mixed in a small test tube. A 0.1 ml aliquot of 4-methylumbelliferyl oleate (0.1 mM, a suspension in McIlvaine buffer) was mixed and incubated at 37°C for 20 min. One ml of 0.1 N HCl was added to terminate the enzyme reaction. After adjusting the pH of the raction mixture to 4.3 by adding 2 ml of 0.1 M sodium citrate, the amount of 4-methylumbelliferone released by the lipase was measured fluorometrically at an emission wavelength of 450 nm and excitation of 320 nm. The effect of each extract was determined by adding 0.05 ml of the extract to the reaction mixture instead of McIlvaine buffer in the foregoing assay system. The activity is expressed as the relative activity to the control.

As shown in Table I, many of the aqueous extracts from medicinal herbs inhibited the lipase activity, in particular Myricae Cortex (*Myrica rubra* Seib. et Zucc.), Anemarrhenae Rhizoma (*Anemarrhena asphodeloides* Bunge.), Nandinae Fructus (*Nandina demestica* Thunb.), Arecae Semen (*Areca catechu* L.), Scutellariae Radix (*Scutellaria baicalensis* Georgi), Angelicae pubescentis Radix (*Angelica pubescens* Maxim.), Eucommiae Cortex (*Eucommia ulmoides* Oliv.), Nomame Herba (*Cassia mimosoides* L. var. *nomame* Makino), Galanga Rhizoma (*Alpina galanga* Swartz), Quercus salicina Suber (*Quercus salicina* Blume), and Theae Folium (*Thea sinensis* L.), which showed a strong inhibitory effect.

The inhibitory effect of these extracts was then examined in the assay system, using triglyceride as a substrate. Each sample for this test was prepared by extracting 5 g of the sample with 100 ml of deioninized water as with the foregoing method, the filtrate being evaporated and then redissolved in 50 ml of deionized water. The assay for lipase activity, using triglyceride as the substrate, was performed according to the method of Fukumoto *et al.*[8] with some modifications. One ml of triolein, 7 ml of McIlvaine buffer (pH 7.4) and 1 ml of the extract or water were mixed in an L-type test tube (ϕ 15 × 180 mm), and 1 ml of the lipase solution (0.7 mg/ml in McIlvaine buffer) was then added before shaking at 37°C for 1 hr. The reaction was stopped by adding ethanol, and the released fatty acids were titrated by 0.05 N NaOH.

Table I. Effect of Aqueous Extracts from Medicinal Herbs on Lipase Activity[a]

Sample	Relative activity (%)	Sample	Relative activity (%)	Sample	Relative activity (%)
Agrimoniae Herba	36	Euchrestae Radix	34	Phellodendri Cortex	86
Akane Radix	18	Eucommiae Cortex	9	Phytolaccae Radix	58
Alpina katumadai Semen	102	Foeniculi Fructus	35	Picrorhiza Radix	35
Armeniacae Semen	115	Galanga Rhyzoma	9	Polygalae Radix	30
Amomi Fructus	46	Gardeniae Fructus	38	Polygoni avicularis Herba	22
Anemarrhenae Rhizoma	3	Gleditschiae Semen	15	Polyporus	140
Angelicae pubescentis Radix	7	Gynostemma Herba	61	Prunus yedoensis Radix	20
Arecae Semen	4	Hoelen	83	Puerariae Radix	73
Asparagi lucidi Radix	41	Hyperici Herba	35	Quercus salicina suber	10
Astragali Radix	19	Isodonis Herba	37	Rehmanniae Radix	71
Aurantii nobilis Pericarpium	100	Kaki Calyx	44	Rhynchophylla	45
Benincasae Semen	100	Kalopanax pictus Radix	22	Sambuci Flos	91
Catalpae Cortex	33	Linderae Radix	19	Sasa Herba	157
Capsici Fructus	29	Liquiritae Radix	65	Scutellariae Radix	6
Carthami Flos	33	Lithospermi Radix	27	Swertiae Herba	34
Cassia Semen	116	Lycii Folium	62	Symphytum Herba	127
Chinae Rhyzoma	29	Magnoliae Cortex	61	Taraxaci Radix	96
Cimicifugae Rhyzoma	37	Meliae Cortex	45	Taxi Folium	25
Clematis Radix	104	Menthae Folium	18	Tetragoniae Herba	21
Cnidii Rhyzoma	14	Mori Cortex	64	Theae Folium	11
Coptidis Rhizoma	21	Myricae Cortex	0.4	Theae Folium (Oolong)	31
Corydalis Tuber	81	Nandinae Fructus	3	Thujopsis dolabrata Herba	15
Cucumis sativus Herba	31	Nomame Herba	9	Trichosanthis Radix	90
Daphniphyllum Herba	59	Nupharis Rhizoma	39	Typha	16
Dianthi Herba	91	Olibanum Resina	65	Viticis trifoliae Fructus	54
Epimedii Herba	25	Paeniae Radix	12	Zedoariae Rhizoma	33
Evodiae Fructus	16	Physalis Radix	154	Zizyphi spinosi Semen	127

[a] 4-Methylumbelliferyl oleate was used as the substrate.

Table II. Effect of Aqueous Extracts from Medicinal Herbs on Lipase Activity[a]

Sample	Addition[b] (mg/tube)	Relative activity (%)
Anemarrhenae Rhizoma	58	157
Angelicae pubescentis Radix	51	74
Arecae Semen	16	32
Eucommiae Cortex	13	90
Galanga Rhizoma	14	35
Myricae Cortex	13	15
Nandinae Fructus	30	74
Nomame Herba	33	41
Quercus salicina Suber	19	64
Scutellariae Radix	18	66
Theae Folium	29	91

[a] Triolein was used as the substrate.
[b] Quantity of extract added to the assay system as dry weight after evaporating.

The results are summarized in Table II. Many of the extracts which showed an inhibitory effect when 4-methylumbelliferyl oleate was used as the substrate also inhibited the lipase activity with triolein as the substrate. However, the extract of Anemarrhenae Rhizoma stimulated the lipase activity when triolein was used as the substrate. The reason for this difference in effect is not clear, but it may be ascribed to an alteration of the emulsion of the substrate (oil) by the extract. Eucommiae Cortex and Theae Folium slightly inhibited the activity, and the other extracts also inhibited the lipase activity in this assay system, Myricae Cortex displaying the strongest inhibitory effect.

These extracts can be expected to prevent undesirable changes in food during storage caused by lipase, and their physiological action be applied.

References

1) K. Taniguchi, C. Ishii, Y. Sunada, and T. Hiramatsu, *Bull. Facul. Agric. Meiji Univ.*, **73**, 9—26 (1986).
2) K. Satouchi, T. Mori, and S. Matsushita, *Agric. Biol. Chem.*, **38**, 97—101 (1974).
3) Y. Garugouri, R. Julien, G. Pieroni, R. Verger, and L. Sarda, *J. Lipid Res.*, **25**, 1214—1221 (1984).
4) D. Lairon, P. Borel, E. Termine, R. Gratarolt, C. Chabert, and L. C. Hauton, *Nutr. Rep. Int.*, **32**, 1107—1113 (1985).
5) T. Horigome, R. Kumar, and K. Okamoto, *Br. J. Nutr.* **60**, 275—285 (1988).
6) C. Ishii, Y. Endo, H. Kimoto, and K. Taniguchi, *Nippon Shokuhin Kogyo Gakkaishi*, **35**, 430—439 (1988).
7) T. J. Jacks and H. W. Kircher, *Anal. Biochem.*, **21**, 279—285 (1967).
8) J. Hukumoto, M. Iwai, and Y. Tsujisaka, *J. Gen. Appl. Microbiol.*, **9**, 353—361 (1963).