

## ON THE METHYL ALCOHOLIC EXTRACT OF DIGENEA SIMPLEX

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The marine plant, *Digenea simplex*, has long been known and used as a folk drug. Its powerful anthelmintic effect and no harmful by-effects have been pointed out previously by several workers (Kanai, Sasaki and Nishimura; Suwa; Hishikawa, and Taki; Koyama; Nunogami; and Fujita).

The author extracted previously a substance containing relatively large amounts of the active components by the following steps: The marine plant—water extract—70% alcohol (ethanol)—98% alcohol (ethanol) extract. Because of the small yield, technical difficulties, and also high cost of alcohol (ethanol), the above method is not so suitable for practical purposes, though useful for research. In this paper, therefore, a simpler methanol extraction method, and some results obtained recently, will be presented.

### METHOD OF EXTRACTION

The fine particles of sand and dust are carefully removed in the same way as was previously employed for alcohol (ethanol) extraction and this clean marine plant (*D. simplex*) is cut up into as small pieces as possible. The material is extracted by boiling with tap water in a water bath, and then filtered through cloth. The filtrates are collected by repeating the extraction on the residue, evaporated and dried on the water bath. To this mass, five to ten volumes of 99% methanol are added and heated under reflux on the water bath. The mixture is centrifuged for 30 minutes at 3000 r.p.m. so that the soluble and insoluble parts are separated. To the insoluble parts, 99% methanol is added again and the soluble parts are again collected. The same technique is repeated until no more soluble parts can be separated out. From the total soluble parts so obtained, the hygroscopic powder, brownish yellow in color, is dried out by heating on a water bath and kept in a vacuum desiccator. The yield of the powder is 2.4% of the marine plant originally used.

### METHOD OF ACTIVITY TEST

Though there are several methods for testing the activity, for example, test of clinical anthelmintic activity, test of worm removing activity in animals, vermifugal activity test *in vitro*, toxicity test on the earthworm, etc., in this work, the author employed the method of using the nerve muscle preparation of *Allolobophora foetida*. This method has the advantage that the test can be performed with minute amounts of the sample and also that the results parallel

comparatively the anthelmintic effect on the human body, particularly of the active components of *D. simplex*.

RESULTS OF ACTIVITY TEST

Besides the component mentioned above, the tests were made on samples which have been extracted from the material, from which fine particles of sand and dust had been removed, directly with 99% methanol either in hot or cold condition,

Diluted solutions (10%–0.001) of each of the above components were pi-petted and dissolved in given ratios in Ringer’s solution in which the nerve-muscle preparation of the earthworm had been suspended. The states of muscular tonus and movement were registered on smoked paper. The mini-mum stimulating concentrations and the grades of activity of the components are shown in Table 1.

The water–99% methanol extract of concentration from 1:100 000 to 1:25 000 showed gradual increase of tonus and of spontaneous movement of

TABLE 1

Extract→ Concen- tration ↓	99% methanol extract from the plant (in hot)	99% methanol extr. from plant (in cold)	Plant—water— 99% methanol extr.	Residue	Material—water extr.—70%— 90% ethanol extr.
500 000			(+)1 (±)2 (–)5		(+)4 (±)8 (–)14
250 000			(+)4 (±)3 (–)7		(+)20 (±)8 (–)7
100 000			(+)11 (–)1 (–)1		(+)3 (±)14 (–)2
50 000		(–)1	(+)2 (±)4 (+)4 (–)1	(–)6	(+)1 (±)5 (–)8
25 000	(+)1 (±)1 (–)2	(+)1 (±)1 (–)3	(+)1 (+)2	(+)1 (±)2 (–)10	(+)7 (±)2 (–)1
10 000	(+)2	(+)1	(+)3	(+)3 (±)2 (±)3 (–)4	(+)6 (±)1
7 500			(+)1	(+)2 (±)3 (±)3 (–)3	
5 000			(+)3 (E-P)	(+)3 (±)7 (+)7 (–)1	(+)3 (E-P)
2 500			(+)4 (E-P)	(+)3 (±)1 (+)1 (–)1	(+)4 (E-P)
1 000	(+)1		(+)1 (E-P)	(+)3	(+)5 (E-P I.N.M.)
750				(+)2 (E-P)	
500			(+)1 (E-P N.M.)	(+)1 (E-P)	(+)4 (E-P I.N.M.)
250			(+)2 (E-P I.N.M.)	(+) (E-P-E)	
100			(+)1 (E-P N.M.)	(+)2 (E-P-E)	(+)1 (E-P N.M.)

Notes. Numbers show the cases occurred.  
E-P; Excitement followed with paralysis.  
N.M.; No movement when rinsed with Ringer.  
E-P-E; Excitement followed with paralysis and the increase of excitement.

muscle. Though this state continued, no paralysis followed. The change was reversed soon to the initial state when the muscle was washed with Ringer's solution.

At concentrations of 1:10 000—1:7 500, the increases in tonus and spontaneous movement were followed by a state of contracture, and no relaxation was observed.

At concentrations of 1:5 000—1:1 000, the contracture occurred the same as above, and was then followed by relaxation; the spontaneous movement decreased gradually and finally ceased. But the movement returned to the initial state, if the muscle was rinsed with Ringer's solution at this time.

At concentrations of 1:500—1:1 000, after rapid increase of tonus, a decrease followed. The spontaneous movement completely ceased and a perfect state of paralysis appeared. Neither relaxation nor movement was observed even if the muscle was transferred into Ringer's solution.

The residue of the extract (water—99% methanol) of concentrations of 1:7 500—1:2 500 produced gradual increase in tonus and spontaneous movement, but no paralysis.

At concentrations of 1:2 500—1:1 000, however, the residue produced increase in contraction, spontaneous movement and a state of contracture afterward, but no relaxation was observed.

At concentrations of 1:750—1:100, the contraction increased than decreased and recovered partly in some cases. Only in one case at a 1:100 concentration, the contracture continued even after the muscle was washed with Ringer's solution.

TABLE 2

No.	Name	Sex	Age	Before of medication		Date of evacuation of worms	Number of worms evacuated	2 weeks after the medication	
				Quality of feces	Number of egg lg.			Quality of feces	Number of eggs lg.
1	M.K.	♀	53	hard	16 100	3rd	1		0
2	Y.K.	♂	38	hard	24 600	2nd 3rd	1 3		0
3	H.T.	♂	61	medium	12 500	2nd	5		0
4	S.O.	♀	40	hard	2 600	2nd	2		0
5	S.Y.	♀	52	hard	9 400	2nd 3rd 8th	2 1 1		0
6	S.S.	♂	57	medium	5 300	5th	1		0
7	T.U.	♂	25	medium	11 900	4th	2	medium	2 600

Notes. Number of patients who evacuated worms/Total patients treated %; 100%.  
Number of patients who lost ascaris-eggs completely/Total patients treated %; 86%.

Total egg reduction/Total of egg counts before treatment %; 96.8%.

Both samples, extracted either in hot or cold conditions directly from the marine plant with 99% methanol, seemed to produce activity in the middle of those of 99% methanol extract and of its residue from water extract,

#### CLINICAL ANTHELMINTIC EFFECT

To the worm infected patients, 1.0 g. of the extract (water—99% methanol) was given on a hungry stomach before breakfast and no food was allowed for 3 hours after treatment. The same treatment was given on the next day so that 2.0 g. was given in 2 days. No purgatives were given in all cases. Stoll's method of counting the egg present was used. The feces were examined at 2 weeks after the treatment. Five slides were prepared from different parts of the feces and the result was considered as 'negative' if no egg was detected. None of the cases showed any harmful by-effects. The worms were voided mostly on the second and third days after medication and with the second day highest. This result agreed with those of Nakao and Suwa.

#### SUMMARY

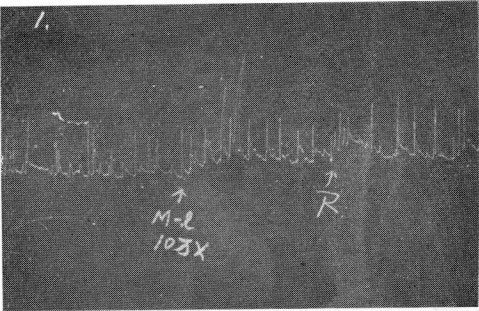
Comparing the reactions well that on the nerve muscle of *Allolobophora foetida*, it was found that of (1) the extract either in hot or cold conditions directly from the marine plant of *Digenea simplex* with 99% methanol; (2) water extract followed with 99% methanol; and (3) residue of (2), (2) showed the highest activity involving excitement in lower and excitement followed by paralysis at higher concentrations. Though this water—99% methanol extract is lower, in activity on the nerve muscle, than one previously reported (the marine plant—water—70% ethanol—98% ethanol), it has the advantage of giving a better yield (2.4% to the original plant and 1% higher than the extract with 98% ethanol), being simpler in extraction technique, and of the lower cost of methanol. This method, consequently, seems to be more suitable for practical and clinical purposes.

In the tests applied on human bodies, though only 7 cases so far, 100% of the patients evacuated worms, 86% showed complete loss of ascaris eggs without any harmful by-effects because of treatment. The good results seem to indicate, that the extract mentioned in this paper is recommendable as an effective anthelmintic.

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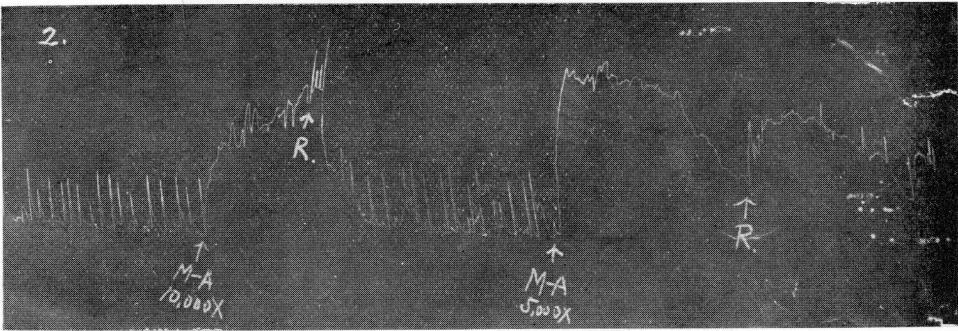
FIG. 1



material—water extr.—  
99% methanol. 1:100 000

Ringer's solution.

2.

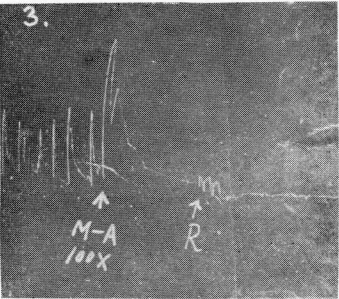


material—water extr.—  
99% methanol. 1:10 000

material—water extr.—  
99% methanol. 1:5 000

FIG. 2

FIG. 3



material—water extr.—  
99% methanol. 1:100.

Takeo Kozawa :

On the Methyl Alcoholic Extract of *Digenea Simplex*

