Volume 08(2) December 2020, 39-46
ISSN 2278- 5906
https://doi.org/10.25173/jtfp.2020.8.2.114
http://www.jtfp.jntbgri.res.in

Journal of Traditional and Folk Practices

Anti photoageing potential of fruits of *Draksha* (*Vitis vinifera* L.) and *Kaashmari* (*Gmelina arborea* Roxb.)

R G Raghi* **and Jollykutty Eapen**

Department of Dravyagunavijnana, Government Ayurveda College,
Thiruvananthapuram - 695 007, Kerala, India
*drraghiraveendran@gmail.com

Received: 14 October 2020 Accepted: 12 November 2020

Abstract

Rasayana chikitsa is one of the eight branches of Ayurveda. *Rasayana* therapy helps in cherishing the wish of human race to achieve long healthy life. Photoageing or skin ageing is one among the wide areas in which *Rasayana* (anti-ageing therapies) can be applied. The present study compares anti photoageing potential of two classical drugs *Kaashmari phala* (Fruit of *Gmelina arborea* Roxb.) and *Draksha phala* (Fruit of *Vitis vinifera* L.). Experiments like DPPH assay, anti-elastase assay and anticollagenese assay were done to substantiate its *Rasayana* action on skin. The results of the studies were analyzed statistically by ANOVA and LSD post hoc pair wise comparison test. Results revealed that fruit of *Kaashmari* showed a greater anti-elastase assay action and anti-collagenese action in comparison to the fruit of *Draksha* ($p<0.001$). The fruit of *Draksha* showed a greater activity in DPPH assay in comparison to the fruit of *Kaashmari*.

Keywords: Anti photoageing, *Draksha*, *Kaashmari*, *Rasayana*

1. Introduction

Ayurveda, the traditional health care system has mainly two aims. One is the preventive aspect to preserve health of those who are healthy and the other one is the curative aspect as treatment for diseases. *Rasayana guna,* which falls under the primary aim for maintaining health and vigour, is defined as the action which wards off ageing and diseases (*yat jara vyadhi nasanam*) (Shailja (Ed), 1999) and this can be correlated with the concept of anti-ageing effect in contemporary science. *Draksha,* one of the extensively used medicinal plant in Ayurveda and in API (Ayurvedic Pharmacopoeia of India) is identified as the dried mature fruits of *Vitis vinifera* L., of family Vitaceae. It has *madhura rasa* (sweet taste), *guru snigdha guna* (heavy and unctuous attribute), *madhura vipaka* (sweet in taste after digestion) and *sheeta veerya* (cold potency) which is similar to that of *Kaashmari phala,* botanically identified as *Gmelina arborea* Roxb., of family Lamiaceae (Sharma and Sharma, (Eds), 1979. According to classics, when there is an unavailability of *Vitis vinifera* L. (VV), *Gmelina arborea* Roxb. (GA) can be used (Chunekar KC (Ed), 2004). VV is an established anti-ageing herb with rich amount of antioxidants like resveratrol. The synonym '*mrdwika*' is explained in most of the classical *nighantus* (Ayurvedic drug lexicons) on emphasizes its action in bringing softness to the skin (Prakash and Harini, 2016). Also, synonyms like *amrita phala, amrita rasa* (like nectar) implies its *Rasayana* property. Similarly, GA possess *Rasayanaguna*. But there is no scientific data proving the same.

Skin ageing is classified as intrinsic (chronological) ageing, which is genetically determined and extrinsic ageing, caused by external factors like UV radiation. Skin that is chronically exposed to sunlight results in photoageing and gets affected by visible pigmentation, elastosis in dermis, atrophy of epidermis, changes in the appearance of collagen and elastin fiber fragmentation (Baumann, 2007). Photoageing has been reported to account for about 70% of the causes of skin ageing (Ichihashi *et al.*, 2009). Collagen, major building blocks

of skin is the main component of connective tissue, hair nails and is responsible for its tensile strength. (Sage and Grey, 1977). Skin elasticity ascribes to elastin fibers. With ageing, collagen and elastin level decreases leading to loss of strength and flexibility of skin. Collagenase is a metalloprotease and elastase is a member of chymotrypsin family. Both of these are primarily responsible for the breakdown of these collagen and elastin fibers (Kim *et al.*, 2004). Finding the inhibitors of elastase and collagenase will be useful not only to prevent skin ageing, but also to maintain the integrity of structures like lung, ligament, cartilage, blood vessel, cornea, intestine, bladder, etc., (Sage and Grey, 1977) and thereby lead to the wider application of the concept of *Rasayana*. The main objective of this study is to evaluate and compare the anti photoageing effect of fruits of VV and GA and thereby introducing an indigenous fruit to public as a nutritive fruit as it has been proven safe (Ashalatha and Sankh, 2014).

2. Materials and methods

2.1. Sample collection

Fruits of *Vitis vinifera* L. (VV) (Fig.1a) were collected from organic farms at Kambam in Tamilnadu and fruits of *Gmelina arborea* Roxb. (GA) (Fig.1c) collected from Government Ayurveda College, Thiruvananthapuram and Tripunithura, Kerala, India. Healthy fruits were washed with water and cleaned. Dried samples were then pounded and stored in air tight containers (Fig.1b & d).

2.2. Sample preparation

2.2.1. *Kashaya* (decoction) preparation (Saastri (Ed), 2013)

Kashaya was prepared according to the procedures explained in *Sarngadhara samhitha*. 48 g each of pounded dried fruit of *Draksha* and *Kaashmari* were taken in different vessels. 192 ml water was poured in both and reduced to 48 ml and strained through clean white cloth (4 layered).

2.2.2. *Sheetha kashaya* (cold decoction) preparation (Saastri (Ed), 2013)

Sheetha kashaya was prepared according to the procedures explained in *Sarngadhara samhitha*. 5 g each of air dried drug was pounded and put in 30 ml of water taken in a beaker. Allowed it to stay for 12 hours and strained through clean white cloth (4 layer).

Fig. 1. Pictures of samples and assay: a. Fresh Sample of *Draksha*; b. Dried sample of *Draksha*; c. Fresh Sample of *Kaashmari*; d. Dried sample of *Kaashmari*; e. Colour change of wells after incubation in anti-collagenase assay

2.3. Experimental analysis

All chemicals were obtained from Sigma-Aldrich Ltd. (Poole, UK) unless otherwise stated.

2.3.1. Anti-collagenase assay

50 mM tricine buffer (400 mM NaCl and 10 mM $CaCl_2$, pH 7.5). Collagenase from was dissolved in the buffer for use at an initial concentration of 0.8 units/ml. The synthetic substrate, FALGPA, was dissolved in the tricine buffer to 2 mM. Sample extracts were incubated with the enzyme in the buffer for 15 min before adding substrate to start reaction. The final reaction mixture (75 µl total volume) contained 25 µl of 50 mM tricine buffer, 25 µl of test extract (20–100 µg/ml) and 25 µl of 0.1 units of enzyme collagenase. Controls performed with 50 mM tricine buffer as test extracts were dissolved in tricine buffer (50 mM), while VVK (*Vitis vinifera* fruit decoction) was used as a positive control because VV is a proven anti-ageing herb. After adding 50 µl of 2 m M FALGPA substrate, collagenase activity was measured immediately at 340 nm (Hua *et al.*, 2008).

2.3.2. Anti-elastase assay

This assay was performed in 0.2 mM tris-HCL buffer (pH 8.0). Porcine pancreatic elastase was dissolved to make a 1 mg/ml stock solution in 0.2 mM tris-HCL buffer. The substrate N-Succinyl-Ala-Ala-Ala-p-nitroanilide (SANA) was dissolved in buffer at 0.8 m M. The test extracts (20-100 mg/ml) were incubated with the enzyme for 20 min before adding substrate to begin the reaction. The final reaction mixture (Total 250 µl) contained 50 µl plant extract, 160µl buffer, 20 µl enzymes, and 20 µl substrate. VVK was used as a positive control. Negative controls were performed using tris-HCl buffer. Absorbance was measured immediately at 410 nm and then continuously for 20 min using a 96 well micro plate reader (David, 1970).

Both the assays were calculated as follows

$$\% \text{ Inhibition} = \frac{(1 - \text{Absorbance}_{\text{Control}}) \text{ X } 100}{\text{Absorbance}_{\text{sample}}}$$

2.3.3. DPPH (2, 2-Diphenyl-1-picrylhydrazil) radical scavenging assay

DPPH is a stable organic radical which has the capacity to scavenge biological reagents. Its solution is deep purple in color with an absorption peak at 517 nm, which disappears with the presence of the radical scavenger in the reactive system, when odd electrons of nitrogen in DPPH molecule are paired. The reactive rate and the ability of the radical scavenger depend on the rate and the peak value of disappearance of the DPPH (Van and Steinbrink, 1981). 2 ml of DPPH radical solution (75 µM) was prepared and 2 ml solutions of plant extract of various concentrations (20 µg/ml–100 µg/ml) were added and ascorbic acid was used as standard. The reaction mixtures were shaken thoroughly and kept at dark for 30 min. Control solution was prepared by adding 2 ml of methanol with 2 ml of DPPH solution. The absorbance of all the reaction mixtures and control solution was measured at 517 nm. The % inhibition was calculated using following formula:

$$\% \text{ Inhibition} = \frac{AC_{517\text{ nm}} - AS_{517\text{ nm}}}{AC_{517\text{ nm}}} \text{ x } 100$$

Where, AC is absorbance of control and AS is the absorbance of sample.

3. Results and discussion

3.1. Results

3.1.1. Anti-collagenase assay

In anti-collagenase assay, collagenase inhibition activity was performed by all the four samples VVK, VVSKS (*Vitis vinifera* L. fruit cold decoction), GAK (*Gmelina arborea* Roxb. fruit decoction) and GASKS (*Gmelina arborea* Roxb. fruit cold decoction) (Fig. 1e and Fig. 2) (Table 1 and 2). Higher inhibition was found in GASKS ($p < 0.001$) it may be due to higher percentage of gallic acid and vit C in GASKS (Raghi and Eapen, 2019).

3.1.2. Anti-elastase assay

In anti-elastase assay, elastase inhibition activity was performed by all the four samples VVK, VVSKS, GAK and GASKS. Classical preparation of decoction (VVK and GAK) showed more elastase inhibition activity than cold decoctions (VVSKS and GASKS). GAK and GASKS shown greater elastase inhibition activity than VVK and VVSKS ($p<0.001$) (Table 3 and 4) (Fig. 3).

Table 1. Anti-collagenase assay results

Sample code	Inhibition rate (%)	Inhibition rate (%)	Inhibition rate (%)	Mean	Standard deviation (±)
VVK-+ve Control	18.84	20.89	18.84	19.60	1.183
GAK	40.49	41.03	40.49	40.67	0.31
GASKS	88.31	84.59	88.31	86.42	2.14
VVSKS	58.88	58.08	58.88	58.47	0.46

Table 2. ANOVA with post hoc comparison among groups

Multiple comparison	Difference mean	SE	P value	P-value interpretation
GAKVVK	-21.07	0.57	<0.001	***
GAKVVSKS	17.8	0.06	<0.001	***
GASKS VVSKS	-27.96	1.03	<0.001	***
GASKS VVK	-66.82	0.52	<0.001	***
VVKVVSKS	-38.87	0.51	<0.001	***
GAKGASKS	-17.8	1.09	<0.001	***

Table 3. Anti-elastase assay results

Sample code	Inhibition rate(%)	Inhibition rate(%)	Inhibition rate(%)	Mean	Standard deviation
VV K-+ve Control	29.65	29.64	29.96	29.75	0.18
GA K	42.65	41.32	42.06	42.01	0.66
GASKS	34.69	35.05	35.04	34.92	0.20
VVSKS	26.61	27.89	28.65	27.71	1.03

Table 4. ANOVA with post hoc comparison among groups

Multiple comparison	Difference mean	SE	P value	P-value interpretation
GAK vs VVK	-12.26	0.34	<0.001	***
GAK vs VVSKS	-14.293	0.25	<0.001	***
GASKS vs VVSKS	-7.21	0.58	0.003	**
GASKS vs VVK	-5.1766	0.02	<0.001	***
VVK vs VVSKS	-14.2933	0.60	0.066	NS
GAK vs GASKS	-7.0833	0.33	<0.001	***

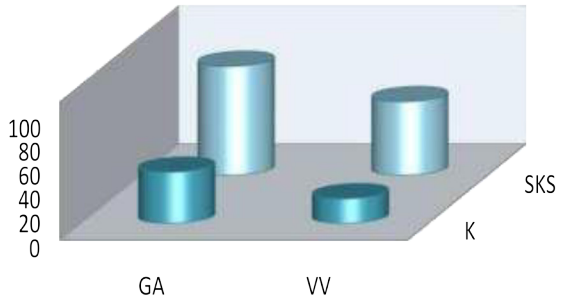

Fig. 2. Anti-collagenase assay

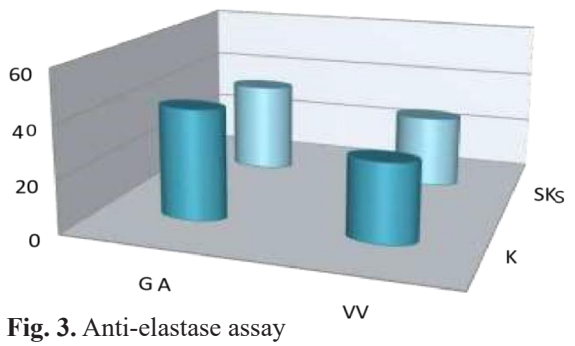

Fig. 3. Anti-elastase assay

3.1.3. DPPH Assay results

In DPPH assay, percentage inhibition of VVK is significantly greater than VVSKS, GAK, GASKS. The inhibition concentration (IC-50) value was determined from extrapolating the graph of percentage inhibition versus the concentration of extract (using linear regression analysis), which is defined as the amount of antioxidant necessary to reduce the initial radical concentration by 50% (Fig. 4). Lower the IC-50 value higher the antioxidant effects. The IC 50 values obtained were GAK- 9.78972 µL, GA SKS- 8.33178 µL, VVK- 4.1424 µL, VVSKS- 4.43188 µL, that is VVK shown less half minimum inhibitory concentration than VVSKS, GAK and GASKS (Table 5 and 6). Which indicates the ability of VV as a potent antioxidant. Among the formulation classical preparation of decoction was more effective as an antioxidant than cold decoctions. Thus, both the drugs GA and VV possess anti photoageing potential in selected parameter.

3.2. Discussion

Rasayana can simply be defined in terms of being the source of 'best quality tissues'. Anti-ageing medicines, immune-modulators, adaptogenes, disease modifying medicines, rejuvenators, health boosting medicines, all these can be clubbed under *Rasayana* group of medicines in contemporary words. Photo oxidative ageing, one among skin ageing is a complex, biological phenomenon. Skin that is chronically exposed to sunlight results in photoageing and gets affected by visible pigmentation, elastosis in dermis, atrophy of epidermis, changes in the appearance of collagen and elastin fiber fragmentation (Baumann, 2007). The photo oxidative ageing is better experimented using the models anti-collagenase assay, anti-elastase assay, antioxidant activities, tyrosinase inhibition assay, hyaluronidase inhibition assay, etc. Among this feasible and more valuable anti-collagenase, anti-elastase and DPPH assays were done.

The samples for the assays were prepared from two types of decoction preparations as explained in a distinguished book of pharmaceuticals, *Sarngadara Samhita*. Wherein it is mentioned that, cold infusion preparation of VV, is used in many diseases like gastritis, acidity, oral thrush and also it is a good laxative and diuretic (Sharma, 2004). It implies that, some constituents in VV may be unstable in excess heat. As GA is VV's substitute having various comparable constituents (Raghi and Eapen, 2019) (Table 7), it may also have such heat unstable compounds. To ensure the type of preparation better go with antiaging potential, two types of decoction preparations for VV and GA were chosen. *Vitis vinifera* L. is a popular edible fruit. In classics, when there is an unavailability of quality fruits of VV, GA fruits are advised to substitute in formulations (Chunekar KC (Ed), 2004). GA is one among the three sweet fruits (*Draksha- Vitis vinifera* L.*, Kharjura - Phoenix dactylifera* L.*, Kaashmari - Gmelina arborea* Roxb.*)* as explained in *Dhanvantari Nighantu* (Sharma (Ed). Dhanvantari 2002). Although *Susrutha,* a renowned Ayurveda physician describes anti-ageing effect of fruits of *Gmelina arborea* L. in *Chikitsa Sthana,* "*Sarvopaghatha Shamaneeyam Rasayana"* chapter public is not aware of its edibility. In Africa, GA is used as a nutritious animal feed (Little, 1983). GA is one of the rich sources of vitamins (Riboflavin, Thiamine, Niacin, Ascorbic acid), amino acids and minerals (Raghi and Eapen, 2019). As GA is an indigenous evergreen plant with a noticeable amount of yearly yield, preservation and promotion of its fruit as a dry fruit will enhance gross cultivation of this tree, which in turn provides pesticide free nutritious fruits. Cultivation of this tree also serves the purpose of producing quality ayurvedic medicines, as GA is one among *Dasamoola* (conventionally used ayurvedic plants with root bark as useful part). In classics, consumption of its dried fruit cooked in milk is good for IUGR (Intra Uterine Growth Retardation) (Chunekar KC (Ed), 2004). India, which is much affected from IUGR (Murki and Sharma, 2014) and this tree which is easily cultivated and found mostly in forest will be easily accessible and be a cost-free medicine. As the tree is one among *Dasamoola,* there is a misconception that root alone is medicinal. Hence awareness has to be created among people for considering the fruit of GA as edible. *In vitro* anti-ageing studies on GA have not been reported yet. Therefore, this study will pave the way for further experimental studies, and may establish formulation prepared out of GA as a potent anti-ageing product. This information may lead to the production of anti-ageing cosmeceuticals from GA.

Journal of Traditional and Folk Practices
Volume 08 (2) December 2020, ISSN 2278-5906

Table 5. DPPH assay ascorbic acid standard

	Concentration (µg/ml)									
	12.5		25		50		100		200	
Control	Absorbance	Inhibition rate (%)	Absorbance	Inhibition rate (%)	Absorbance	Inhibition rate (%)	Absorbance	Inhibition rate (%)	Absorbance	Inhibition rate (%)
	1.4044	21.90	1.0782	40.04	0.7121	60.40	0.2921	83.75	0.0692	96.15

Table 6. DPPH assay sample

					Concentration (µL)					
	1.25		2.5		5		10		20	
	Absorbance	Inhibition rate (%)	Absorbance	Inhibition rate (%)	Absorbance	Inhibition rate (%)	Absorbance	Inhibition rate (%)	Absorbance	Inhibition rate (%)
GAK	0.2697	17.04	0.2104	35.28	0.1750	46.17	0.1363	58.07	0.1004	69.12
GASKS	0.2475	14.64	0.2050	23.87	0.1761	36.94	0.1211	45.83	0.0861	62.75
VVK	0.2228	31.47	0.1707	47.49	0.1339	58.81	0.0870	73.24	0.0621	80.9
VVSKS	0.2397	26.27	0.1848	43.16	0.1567	51.80	0.0911	71.98	0.0791	75.67

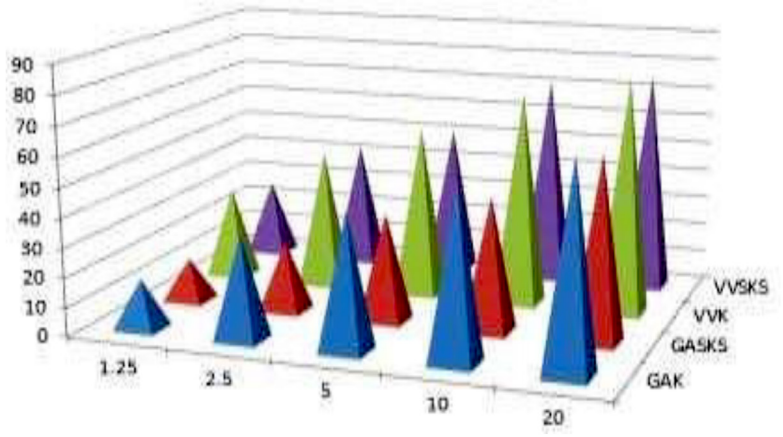

Fig.4. DPPH assay IC 50 value

44

Table 7. Constituent profiling

Sl. No.	Amino acids	GA (dried fruitmg /100g protein)	VV (Percentage free amino acid in grape juice)
1.	Lysine	3.66	0.3
2.	Threonine	2.05	2.3
3.	Cysteine	2.64	-
4.	Methionine	3.81	0.3
5.	Isoleucine	3.08	1.1
6.	Leucine	4.13	1.3
7.	Tyrosine	4.23	0.2
8.	Phenylalanine	3.41	0.6
9.	Arginine	4.29	-
10.	Histidine	2.96	1.4
Vitamins/100g			
11.	Vit C	22.88mg	10.8 mg
12.	Vit A	-	66 IU
13.	Vit E	-	0.19 mg
14.	Vit K	-	14.6 µg
15.	Riboflavin	0.30mg	-
16.	Thiamin	0.64mg	-
17.	Niacin	0.64mg	0.188 mg
Minerals (mg/100g of fruit)			-
18.	Zinc	1.54	0.07
19.	Copper	0.47	0.12
20.	Iron	5.71	0.36
21.	Magnesium	32.9	7
22.	Calcium	22.77	10

4. Conclusion

The present study was undertaken with an aim of proving anti photoageing potential of both fruits of *Kaashmari* (GA) and *Draksha* (VV) using various parameters. In which, fruit of *Kaashmari* showed greater anti collagenase and anti-elastase action in comparison to the fruit of *Draksha* ($p<0.001$). Fruit of *Draksha* showed greater activity in DPPH assay in comparison to the fruit of *Kaashmari*. Thus both drugs showed anti photo ageing potential. Apart from this, fruits of *Kaashmari* can be popularized for its edible value.

Acknowledgements

The authors would like to thank Principal, Government Ayurveda College, Thiruvananthapuram and Biogenix Research Centre, Poojapura for providing necessary facilities.

References

Ashalatha M and Sankh K. 2014. Toxicity study of *Gambhari Phala Churna*. Int. Ayurveda Med. J. 2(6): 959-963.

Baumann L 2007. Skin aging and its treatment. J. Pathology. 211(2): 241-251.

Chunekar K C (Ed). Bhavamisra 2004. *Bhavaprakasa Nighantu*. Chaukhambha Bharati Academy, Varanasi. pp. 127, 243.

David M Shotton 1970. Elastase. Methods in Enzymology. 19: 113-140.

Hua L, Xiaoyu W, Peihong L, Yong L, Hua W 2008. Comparative Study of Antioxidant Activity of Grape (*Vitis vinifera*) Seed Powder Assessed by Different Methods. J. Food and Drug Analysis. 16(6): 67-73.

Ichihashi M, Ando H, Yoshida M, Niki Y and Matsui M 2009. Photoaging of the skin. J. Antiaging Med. 6: 46-59.

Kim Y, Uyama H, Kobayashi S 2004. Inhibition effects of (+)-catechin-aldehyde polycondensates on proteinases causing proteolytic degradation of extracellular matrix. Biochem. Biophys. Res. Commun. 320: 256-261.

Little E L 1983. Common fuelwood crops: A Handbook for their identification. Mcclain printing co, parsons, WV. pp. 21-32.

Murki S and Sharma D 2014. Intrauterine growth retardation - a review article, J. Neonatal Biology. 3(3).

Prakash L Hegde and Harini A 2016. A text book of *Dravyaguna Vijnana*. Choukambha Publisher, New Delhi. 1: pp. 179.

Raghi R G and Eapen J 2019. Comparative phytochemical analysis of fruit of *Draksha* (*Vitis vinifera* L.) and *Kaashmari* (*Gmelina arborea* Roxb.). Int. J. Ayush 8 (2): 21-35.

Saastri P P (Ed) *Sarngadhara* 2013. *Sarngadhara Samhitha*. Chaukhambha publication, New Delhi. pp. 144, 212, 306.

Sage E H and Gray WR 1977. Evolution of Elastin structure. Adv. in Exp. Med. Biology. 79: 291-312.

Shailja S (Ed) *Sarangdhara* 1999. *Sarangdhara Samhita*. Chukhambha Orientalia, Varanasi. 1: pp. 33.

Sharma P V (Ed) *Dhanvantari* 2002. *Dhanvantari Nighantu*. Chaukhamba Orientalia, Varanasi. pp. 36-37.

Sharma P V 2004. Classical uses of medicinal plants. Chaukhambha Viswabharati, Varanasi. pp. 197-200.

Sharma P V and Sharma G P (Eds) Kaiyadeva 1979. *Kaiyadeva Nighantu*. Chaukhambha orientalia, Varanasi. pp. 9.

Van Wart H E and Steinbrink D R 1981. A continuous spectrophotometric assay for *Clostridium histolyticum* collagenase. Anal. Biochem. 113: 356-365.