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Ethnobotanical survey on distribution of medicinal plants in the genus *Arisaema* in ruins of fortresses used in medieval Japan

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Members of the genus *Arisaema* (Araceae) are perennial understory herbs that were traditionally used in medieval Japan in the treatment of flesh wounds resulting from warfare to prevent tetanus. This study aimed to elucidate the distribution of *Arisaema* in the ruins of fortresses dating from the medieval period in Japan. Two hypotheses were tested: that *Arisaema* are commonly found in the ruins of fortresses in central Japan and that *Arisaema* grows more intensively at military sites than in surrounding areas. *A. serratum* var. *serratum*, *A. thunbergii* subsp. *urashima*, *A. yamatense* subsp. *yamatense*, *A. kishidae*, and *A. tosaense* were observed at 19 of the 27 fortresses examined. A census conducted along the major traditional routes in northwestern Nara Prefecture revealed that *Arisaema* was clumped more intensely at military sites, and fewer plants were observed in the surrounding areas. These findings indicate a strong association between *Arisaema* and military sites. Climatic conditions and modification of soil microclimate resulting from fortress construction could not adequately explain this association. However, this association, combined with the historical importance of *Arisaema* in the treatment of wounds, implies that *Arisaema* plants found in the fortresses are historical remnants of plants used to treat warriors and commanders in medieval Japan.

Key words: *Arisaema*, commander, ethnobotany, medieval Japan, military, tetanus.

INTRODUCTION

The genus *Arisaema* (Araceae) contains about 180 species of perennial understory herbs that mainly grow in the evergreen and deciduous forests of Asia, as well as some forests in East Africa and North America. At least 35 species, including 22 subspecies and varieties, have been documented in Japan (Gusman and Gusman, 2006; Murata, 2011).

The tubers or rhizomes of some *Arisaema* plants have traditionally been used in Asia for their analgesic, antitumor, and pesticide properties, and also against snakebite and tetanus (Jiangsu Xin Yixue Yuan, 1985). In Japan, medicine for treating open wounds came to prominence from the 14th to 16th centuries, when Japan was embroiled in protracted wars on a national scale (Goble, 2005). Use of *Arisaema* ("*tennansho*" in Japanese) for stopping blood flow and protecting against

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tetanus was reported in Japanese medical literature from the 14th century, e.g., "*Man'anpo*" by Seizen Kajiwara (Kajiwara, 1327) and "*Yurin's Fukudenho*" by the Buddhist monk Yurin (Yurin, mid-14th century). Recent studies have shown that the members of the genus are rich in the triterpenoid saponin, benzoic acid, alkaloids, and lectins. For example, extracts from *A. flavum* (Singh et al., 2004), *A. tortuosum* (Dhuna et al., 2005), and *A. decipiens* (Zhao et al., 2010) all exhibit antitumor effects and suppress cancer cell growth, while extracts from *A. flavum* exhibit antibacterial effects (Bibi et al., 2011).

Arisaema plants are naturally found on disturbed open sites such as volcanic zones, scree slopes along a stream, cliffs that easily collapse, and silviculture forests in Japan (Hotta, 1974; Gusman and Gusman, 2006; Murata, 2011). While some specimens have been documented from the territory of forts, e.g., *A. nambae*, which was first collected from Bitchu-Matsuyamajo fortress in 1952 and described then as a novel species (Kitamura and Murata, 1966), and *A. kishidae* and *A thunbergii subsp. urashima* from Shigisanjo fortress (Koshimizu and Iwata, 1976). Several thousand medieval fortresses are scattered throughout Japan. Most were constructed on mountain ridges or at the tops of hills due to the advantage that these locations offered for surveillance of the surrounding area and for protection, as described in the *Nihon Jokaku Taikei* (e.g., Murata, 1980). The location of these forts and modification of the soil microenvironment during construction may have provided favorable conditions for the growth of these plants. However, there have been no reports focusing on the relationship between distribution of *Arisaema* and ruins of fortresses.

The present study aimed to elucidate the distribution of *Arisaema* in the ruins of fortresses dating from the medieval period in Japan. Two hypotheses were tested: (i) *Arisaema* is commonly found in the ruins of fortresses in central Japan; (ii) *Arisaema* grows more intensively at military sites compared with surrounding areas such as forests and other traditional construction.

MATERIALS AND METHODS

Study area

To examine the occurrence of *Arisaema* at ruins of fortresses, 26 fortresses in central Japan were selected for this study (Figure 1). These ruins were selected based on historical descriptions of the occupiers, when they were used, and which functional structures remain according to *Nihon Jokaku Taikei*. Although there were numerous other fortresses in the region, many forts were excluded from this study because they had been developed as public parks, schools, or residential areas. Many forts with access routes lost due to dense overgrowth by bushes were also excluded. In this study, each of the selected fortresses was assigned a code consisting of two uppercase letters denoting the prefecture, followed by a number, which corresponded to that used in the *Nihon Jokaku Taikei*. The study area is close to Kyoto, which was the capital city from the late 8th to mid-19th century and served as the seat of power for several successive emperors and generals (shoguns), and which experienced very intense battles during the medieval period. The mean annual temperature and precipitation in the area range from 13 to 16°C and 1,366 to 2,087 mm, respectively. At present, most of the ruins are located in areas that are used for silviculture with Japanese cypress (*Chamaecyparis obtusa*) or Japanese cedar (*Cryptomeria japonica*). Shade-tolerant herbs such as *Rubus buergeri, Corydalis incise,* and *Houttuynia cordata,* and shrubs such as *Aucuba japonica* and *Dendropanax trifidus*, were common. In open areas, *Galium spurium* var. *echinospermon* and *Fallopia japonica* were frequently observed. *Arisaema* spp. censuses were conducted during the flowering season from April to June in 2011–2016. Species identification of the genus *Arisaema* was performed *in situ* based on the morphological descriptions of Gusman and Gusman (2006) and Murata (2011). Some of the specimens were deposited at the herbarium of the Kii-Oshima Experimental Station, Field Science Education and Research Center, Kyoto University.

To compare the distribution pattern of *Arisaema* between military sites and surrounding areas, a precise census along the major traditional routes was conducted to the south of the Ikoma Mountains and Yata Hills, with the principal town situated at 34°37′45″N and 135°42′2″E. This area, which is located on the border of Osaka Prefecture and northern Nara Prefecture (Figure 1), has a rich history spanning ancient to medieval Japan, with extensive information available on the archaeological sites and fortified ruins. The incidence of *Arisaema* plants was observed by counting all individuals located within five meters on either side of footpaths for a total of 98.2 km. Routes comprised mountain ridges and streams, and villages containing 17 Buddhist temples, 19 Shinto shrines, and 9 military ruins (e.g., Uchino, 2015).

Data analysis

A Mann-Whitney *U*-test was conducted to evaluate the effect of mean annual temperature and annual precipitation on the incidence of *Arisaema* according to the method of Sokal and Rohlf (1981). To evaluate the distribution pattern for this plant in forests and villages along the route of the census, a Morisita overlap index ($I\sigma$) was calculated as follows (Morisita, 1959):

$$I\sigma = q\sum x_i(x_i - 1)/\sum x_i(\sum x_i - 1)$$

where x_i is the number of individuals in the ith sample unit (i = 1, 2, 3, ⋯, q) and q is the number of sample units. In this research, a sample unit is defined as being a 100-m section of footpath including a 5-m area adjacent to each side of the path (sample unit area: 1,000 m^2). Values of $I\sigma < 1$ indicate a uniform, $I\sigma = 1$ a random, and $I\sigma > 1$ a clumped distribution. Significant deviations of $I\sigma$ from 1 were assessed using a chi-squared analysis as follows (Krebs, 1998):

$$\chi^2 = I\sigma\,(\sum x_i - 1) + q - \sum x_i$$

with v = q – 1 degrees of freedom.

A 2×2 contingency chi-squared analysis was performed for comparison of *Arisaema* incidence at military and other sites.

RESULTS

Arisaema in fortress ruins in central Japan

Arisaema plants were found in 19 of the 27 surveyed fortress ruins in central Japan (Figure 1). The species identified and the climatic conditions at each of the fortresses are summarized in Table 1. *A. serratum* var.

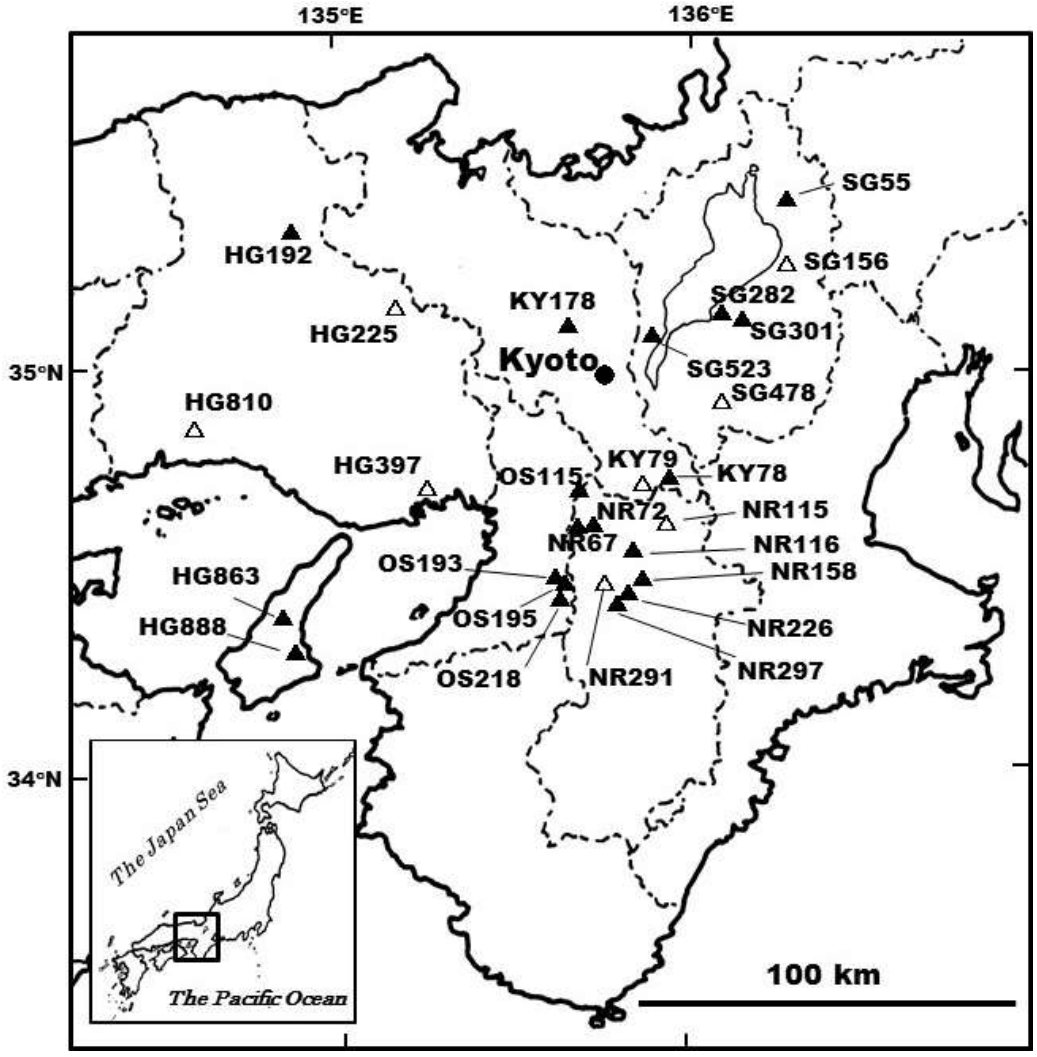

Figure 1. Distribution of *Arisaema* in the ruins of medieval fortresses in central Japan. Solid and open symbols indicate whether *Arisaema* was observed or not observed, respectively. The inset shows the location of the study area in the Japanese archipelago.

serratum, *A. thunbergii* subsp. *urashima*, and *A. yamatense* subsp. *yamatense* were the most widely and frequently observed species (Figure 2). On the other hand, dense populations of *A. tosaense*, *A. kishidae*, and a form of *A. serratum* var. *serratum*, previously called *A. longilaminum*, were observed at sites HG863, NR67, and NR116, respectively. Most of these species were easily identified by conspicuous morphological characters except for *A. kishidae*, which resembles *A. undulatifolium*. The two species could be distinguished based on morphological characters of the female plants; the length of the peduncle was shorter than the petiole in females from site NR67, which were similar to *A. kishidae*, and longer in the *A. undulatifolium* group (Murata, 1986). In addition, the number of ovules per ovary in plants at NR67 (*A. kishidae*) was in the range 3–5, which was fewer than in the *A. undulatifolium* group, which typically has more than ten (Murata, 1986).

Interestingly, although the fortresses OS193 and OS195 were 1 km apart, both supported patches of *A. serratum* var. *serratum*, even though no individual plants were observed along the footpath between the two sites. Analysis by a Mann–Whitney *U* test showed that neither the average annual temperature ($U = 86$) nor the annual precipitation ($U = 94$) significantly affected the incidence of *Arisaema* plants ($P > 0.05$).

Distribution pattern for *Arisaema* along the census route

Figure 3 shows the *Arisaema* distribution observed along the route of the census in the southern Ikoma Mountains and Yata Hills. In the Ikoma Mountains, dense populations

Table 1. Distribution of *Arisaema* in ruins of medieval fortresses in central Japan.

Fortress Code	Commanders/Occupiers	Mean annual temperature (°C)	Mean annual precipitation (mm)	*Arisaema* species observed
HG192	Akamatsu family	14.2	1738	*A. serratum* var. *serratum*
HG225	Akamatsu family	14.6	1634	NF
HG397	Akamatsu family	15.7	1400	NF
HG810	Akamatsu family	15.2	1366	NF
HG863	Atagi Family	15.8	1439	*A. tosaense*
HG888	Atagi family	15.8	1439	*A. thunbergii* subsp. *urashima*
KY78	Emperor Go-Daigo	12.9	1776	*A. yamatense* subsp. *yamatense*
KY79	Kizu family	15.0	1540	NF
KY178	Mitsuhide Akechi	15.4	1730	*A. serratum* var. *serratum, A. yamatense* subsp. *yamatense*
NR67	Hisahide Matsunaga	14.8	1585	*A kishidae, A. serratum* subsp.. *serratum, A. thunbergii* subsp. *urashima*
NR72	Tsubai family, H. Matsunaga	14.8	1585	*A. thunbergii* var. *urashima*
NR115	Yamada family	14.0	1772	NF
NR116	H. Matsunaga	14.0	1772	*A. serratum* var. *serratum*
NR158	Akiyama family	13.9	1934	*A. yamatense* subsp. *yamatense*
NR226	Warrior monks	15.2	1786	*A. serratum* var. *serratum, A. thunbergii* subsp. *urashima, A. yamatense* subsp. *yamatense*
NR291	Ochi family	15.1	1865	NF
NR297	Ochi family	15.1	1865	*A. yamatense* subsp. *yamatense*
OS115	Nagayoshi Miyoshi	14.2	1624	*A. thunbergii* subsp. *urashima*
OS193	Masashige Kusunoki	14.6	1781	*A. serratum* var. *serratum*
OS195	M. Kusunoki	14.6	1781	*A. serratum* var. *serratum*
OS218	M. Kusunoki	14.6	1781	*A. yamatense* subsp. *yamatense*
SG55	Nagamasa Azai	14.4	2087	*A. thunbergii* subsp. *urashima*
SG156	Mitsunari Ishida	14.4	2087	NF
SG282	Nobunaga Oda	14.6	1718	*A. thunbergii* subsp. *urashima*
SG301	Rokkaku Sasaki	14.6	1718	*A. serratum* var. *serratum*
SG478	Mikumo family	14.4	1644	NF
SG523	Nagamasa Azai	14.8	1719	*A. yamatense* subsp. *yamatense*

Fortress codes are the same as in Figure 1 (as shown in the text). Climate data from http://ja.climate-data.org/location/. NF: not found.

of *A. thunbergii* subsp. *urashima* were observed in the NR67, F3 (Akasakajo) and F4 (Hanaharajo) fortresses, as well as several religious sites in their vicinity. These species were also found at the ancient fortress of F1 (Takayasujo), which was constructed in the late 7th century. The distribution of *A. kishidae* was limited at NR67, F4, and R1 (Takayasujyo Kuraato), which are considered to be ruins of food depots associated with fortress F1. A few individuals of *A. serratum* var. *serratum* were occasionally found on ridges of the Ikoma Mountains near the border between Osaka and Nara Prefectured.

Conversely, few *Arisaema* plants were found in the Yata Hills. Dense but restricted populations of *A. thunbergii* subsp. *urashima* were found at R2 (Byodoji Yakata), which is considered to have been the dwelling of local commanders, and also at religious sites at the entrance of NR72 (Tsubaijo). In addition, a small population of *A. thunbergii* subsp. *urashima* was observed on a scree slope along a stream approximately 2 km north of R2.

Table 2 shows the results of the route census and the Morisita overlap index. On all of the routes along ridges, streams, and villages in the Ikoma Mountains and the Yata Hills, the values of $I\sigma$ were significantly greater than 1, indicating that the *Arisaema* plants were distributed in clumps. In streams, the clumps of *Arisaema* were composed largely of *A. kishidae*, which grew in fortress NR67 and in a stream next to the ruin of depot R1. Along the ridges, *A. thunbergii* subsp. *urashima* was dominant, especially at NR67, F1, F2, and F3 and the religious sites close to these fortresses. Consequently, the 2×2 contingency chi-squared analysis revealed that *Arisaema* plants were more frequently observed at military sites than at other sites ($\chi^2 = 149.0$, $P < 0.01$).

DISCUSSION

The first hypothesis, that *Arisaema* is commonly found in

Figure 2. *Arisaema* growing in the ruins of medieval fortresses in central Japan (a), *A. serratum* var. *serratum* with white stripes on spathe and capitate spadix appendage in OS193; (b) *A. yamatense* subsp. *yamatense* with rounded, green, glossy apex of the spadix appendage and (d), ripening of the spadix in NR297; (c), *A. thunbergii* subsp. *urashima* with a prolonged spadix appendage in HG888; (e), *A. kishidae* with comparatively shorter peduncle than petiole in female plant in NR67; (f), *A. tosaense* with green body and long caudate spathe limb in HG863.

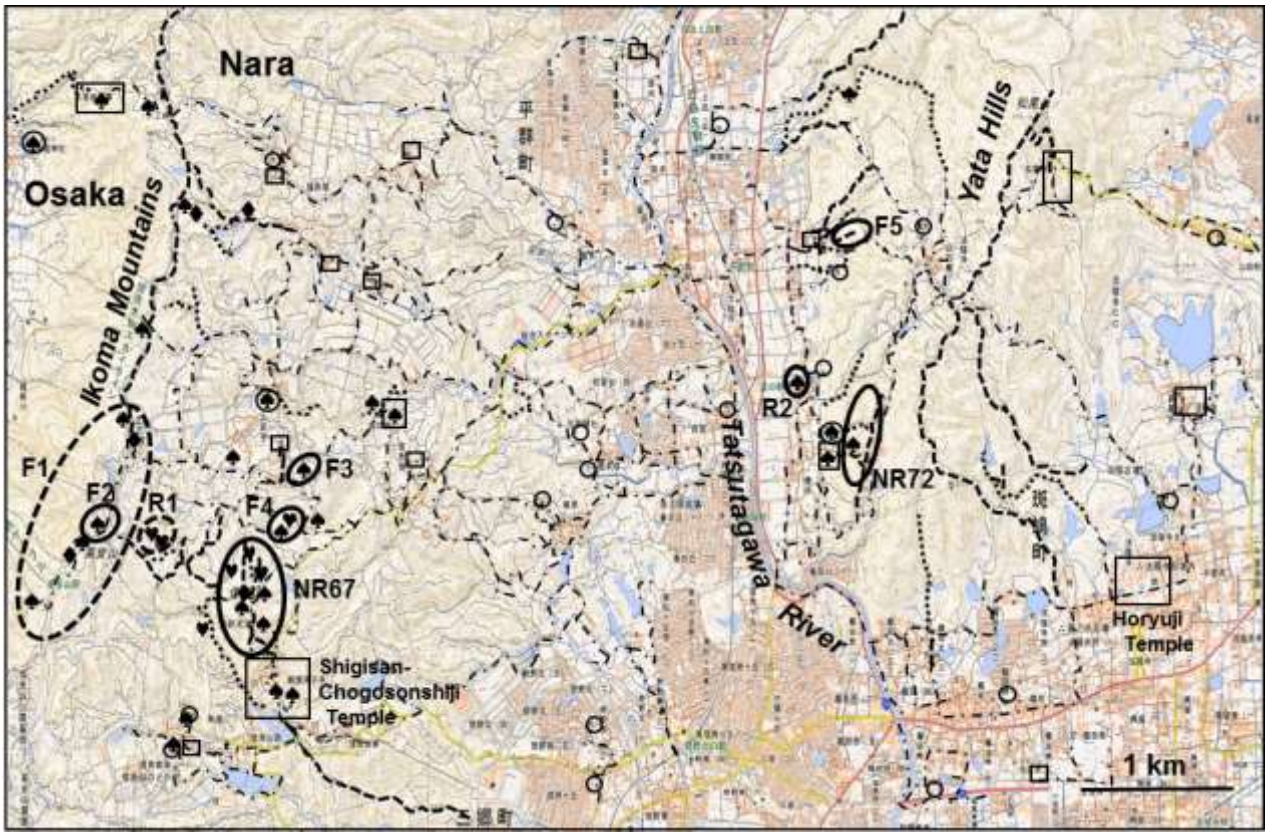

Figure 3. Map of route census showing the distribution pattern for *Arisaema* plants in the southern Ikoma Mountains and Yata Hills (basic map obtained from the Geospatial Information Authority of Japan, https://maps.gsi.go.jp/). The survey route is shown as a broken line (ridge), dotted line (stream), and dash-dotted line (village). Bold ellipses, rectangles and circles indicate military sites, Buddhist temples, and Shinto shrines, respectively. Symbols indicate *A. kishidae* (♥), *A. serratum* var. *serratum* (♦) and *A. thunbergii* subsp. *urashima* (♠).

Table 2. Distribution pattern of *Arisaema* spp. observed during a route census in the Ikoma Mountains and Yata Hills.

Region	Part	No. of quadrats examined	No. of quadrats *Arisaema* observed	Total no. of individuals	*Iσ*	χ2	*P*
Ikoma Mtns.	Ridge	83(15)	18 (9)	420 (285)	11.4	3145	< 0.01
	Stream	32(6)	11 (5)	230 (180)	6.53	262	< 0.01
	Village	502(1)	15 (1)	350 (40)	47.5	18114	< 0.01
Yata Hills	Ridge	84(12)	2 (1)	25 (5)	56.0	1403	< 0.01
	Stream	42(0)	1 (0)	20 (0)	25.4	505	< 0.01
	Village	239(1)	1 (1)	30 (30)	239	7140	< 0.01
Total		982(35)	48(17)				
	Contingency analysis of *Arisaema* incidence on military sites and other sites:					χ2 = 149.0,	*P* < 0.01

Each quadrat was defined as a 100 m-section of footpath with a 5-m area adjacent to each side of the path (sample unit area: 1,000 m^2). *Iσ*: Morisita overlap index. Parentheses indicate corresponding number at each military site.

the ruins of fortresses scattered throughout central Japan, was supported. Although several thousand medieval fortresses are scattered throughout Japan and the fortresses surveyed here are merely a representative sample, this study provided preliminary evidence of a relationship between the incidence of *Arisaema* and fortresses. The second hypothesis, that *Arisaema* grows more intensively at military sites compared with surrounding areas such as natural forests and other traditional construction, was also supported by the route census of this study. These results thus indicate a strong association between *Arisaema* and military sites. This finding also predicts target sites to find this plant.

Why is this plant commonly found at military sites? The establishment of *Arisaema* populations is highly dependent on several environmental conditions, especially insolation and soil moisture conditions (Gusman and Gusman, 2006). The cutting of trees, leveling of the ground, digging, and occasional stone wall construction involved in fortress construction would have affected the solar radiation reaching the forest floor and the drainage characteristics of the soil, and consequently, may have provided conditions favorable for the growth of these plants. Similar modifications to the microenvironment are expected to have occurred during construction of Buddhist temples and Shinto shrines. However, in the route census in this study, which included 17 Buddhist temples and 19 Shinto shrines, *Arisaema* was typically only found at religious sites near forts. This observation does not adequately support the idea that a change in soil microclimate resulting from fortress construction was a major factor in why these plants were found within forts.

On the other hand, it is worth considering the bibliographic information about medicinal use of *Arisaema* in medieval Japan. In an anonymously authored document (Anonymous, mid-14th century), the "*Kinso Ryoji Sho*", which dealt with emergency treatment of wounds resulting from weapon injuries, *Arisaema* is recommended for treating the following: stopping blood flow from a wound; returning viscera to the abdominal cavity; treating wounds by everting them slightly to expose the damaged internal tissue so that it can be cleaned and medicines can be applied to prevent death by septicemia; treating brain tissue protruding from head wounds; as well as other injuries. During conflicts with rival chiefs, trade routes were severed, and warriors and commanders would need to procure their own medicine for use on the battlefield (Shinmura, 2013). It is very likely that *Arisaema* would have been stored or cultivated in the vicinity of warrior residences and military facilities. Indeed, even after the national wars ended in the early 17th century, central and local administrations established by warriors managed their own medicinal herb gardens and frequently cultivated *Arisaema* (Ueda, 1930).

Taken together, the incidence of *Arisaema* in the ruins of military sites, combined with the historical importance of *Arisaema* in the treatment of wounds resulting from warfare, strongly suggest that the *Arisaema* plants found in the fortresses are historical remnants of the plants that were used to treat warriors and commanders. In this context, to fully appreciate the distribution and regional diversity of this plant species in Japan, further studies from an ethnobotanical perspective are necessary.

Conclusion

This study revealed a strong association between the incidence of plants in the genus *Arisaema* and the ruins of medieval fortresses in central Japan. To better appreciate the distribution of this genus and its regional diversity in Japan, an ethnobotanical survey and bibliographic data search are considered essential.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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