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Valorization of Bio-Resources in Semi-Arid and Arid Land

- Functional Analysis Group of STREPS Project in Tunisia -

Junkyu HAN[1] and Hiroko ISODA*[1]

Abstract: Olive, halophyte and aromatic plants are undeniable natural resources especially in semi-arid and arid land of Tunisia. In this research, we used the multifaceted bioassay and the molecular biology approaches in valorizing medicinal plants, proteomics and genomics in particular, to decipher the specific signal transduction that occur in mammalian cells following treatment with components or extract from olive, halophyte and aromatic plants. The molecular biology approaches provide reliable and quick results in the determination of the effect of components or extract from olive, halophyte and aromatic plants at the molecular level in mammalian cells to complement the known traditional uses of olive, halophyte and aromatic plants. Molecular biology approach, therefore, provides opportunities for the establishment of scientific evidence regarding the medicinal properties or therapeutic use of olive, halophyte and aromatic plants.

Key Words: Aromatic plants, Halophytes, Molecular biology approach, Multifaceted bioassay, Olive

1. Introduction

This project targets the arid land in Tunisia in northern Africa, and we will deploy the investigation of useful bio-resources based on food culture and traditional medicinal information, investigation of vegetation distribution, investigation of temperature/soil saline characteristics that are factors of environmental deterioration in arid land, investigation of habitat environment, perform exhaustive component analysis and functionality analysis of bio-resources that were selected based on these investigations, and create a database that encompasses the species/habitat environment information and functionality/element/chemical compound information of arid land bio-resources, as well as a barcode management library of seeds and functional elements. Furthermore, the project will develop the technologies for cultivation/breeding methods that aim at adapting to the environment, and will seek a high-degree utilization of arid land bio-resources by introducing food utilization and processing technologies.

The characteristics of the northern African region is that unlike the continental type of arid land, the distance between the Mediterranean Sea and the Sahara Desert is short, resulting in a large aridity gradient that produces a unique biodiversity. It is the only holarctic floral region in the Gondwana land (South America / Africa / Australia / India / Antarctica), and unique flora exist from the plant geography point of view as well. We are investigating useful biological activities from the traditional medicinal plants in North African region.

We seek anti-oxidative activities from local plant resources which have been used as traditional medicines. Plants that grow on arid land possess functions that protect them from the stress of harsh habitat, and it is thought that they contain unique functional components that play such roles. To take an example from recent studies, it has been reported that olives and medicinal plants that grow in Sahara desert oases contain antioxidant materials (such as polyphenol) that are several times higher than those found in species growing in the Mediterranean region (Omri *et al*., 2010; Han *et al*., 2009a; Abaza *et al*., 2008) and that these materials possess antitumor activity, anti-allergic activity, neuronal cell death repressor activity, skin function activity, etc (Villareal *et al*., 2010; Han *et al*., 2009b; Omri *et al*., 2009; Matsuyama *et al*., 2009; Kawano *et al*., 2009; Han *et al*., 2007; Yamada *et al*., 2008; Kashiwagi *et al*., 2009).

From aromatic plants and olives, we also explore for bioactivities effective against various diseases such as allergy, nervous disease like Alzheimer's disease, and cancer. In order to elucidate molecular mechanisms of their action, fractionation of the plant extracts and identification of the active compounds are performed. Moreover, we investigate their effect on intracellular signal transduction systems related to the diseases.

2. Materials and Methods

To evaluate the functional molecular of bio-resources, the various methods were carried out. First, we used the bioassays to perform the screening of bioactivity of

* Corresponding Author: isoda.hiroko.ga@u.tsukuba.ac.jp

Tennoudai 1-1-1, Tsukuba city, Ibaraki, Japan

1) Alliance of Research on North Africa, University of Tsukuba

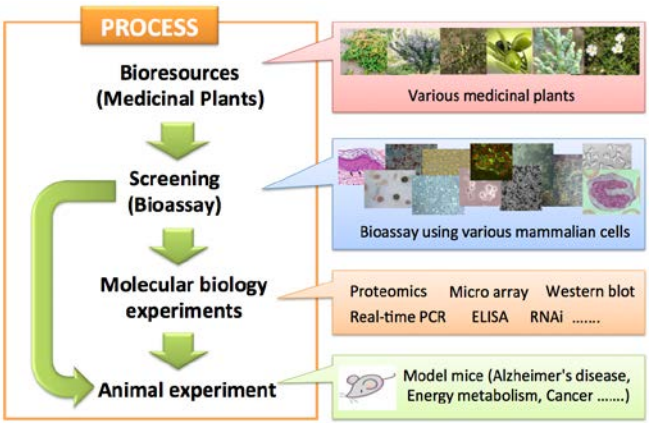


Fig. 1. The process of functional analysis from bio-resources.

bio-resources. We performed the basic assay (DPPH assay, Total polyphenol assay, MTT assay, LDH assay, Intracellular ROS assay, Intracellular F-actin assay, Intracellular ATP assay), Neuronal assay (Acetylcholinesterase assay, Neurite outgrowth assay, Neuroprotection assay), Intestinal assay (TER assay, α-Glucosidase assay, Cholesterol assay, Epithelium permeability assay), Immunity assay (β-hexosaminidase assay, Histamin assay, Intracellular Ca ion assay, Immunoactivity assay), Anti-cancer assay (Leukemia differentiation assay, Cancer migration assay), Lifestyle-related disease assay (Adipogenesis assay, Adiponectin assay, ACE II assay, Alkaline phosphatase activity assay, Stress recovery assay), Skin assay (Melanogenesis assay, Dermal papilla growth assay, Epithelium permeability assay) to find a new functional molecular from olives, halophytes and aroma plants (**Table 1**).

After bioassay, we performed the molecular biology experiments to make clear the mechanism of these effects from olives, halophytes and aroma plants. We carried out the western blotting, Real time-PCR, Micro array, Cell cycle, Proteomics, DNA fragmentation test, Immunostaining, ELISA to evaluated the effect of functional molecular on proteins and genes expression (**Table 2**).

3. Results and Discussion

In this year, we investigated the 10 varieties of olive, 12 species of halophyte and 13 species of aroma plant (**Table 3**).

The traditional usage information of Tunisia was contributed to obtain the positive results on screening using various bioassays. We found that the olive extracts and its components have anti-oxidant, anti-allergy and anti-cancer effects. Some of halophyte extracts have anti-oxidant, anti-cancer, anti-fat and activation of energy metabolism effects. The some of aroma plant extracts and its components have anti-oxidant, anti-stress and melanogenesis regulation effects (**Table 4**). We expect that these finding will be a basic

Table 1. The bioassay list for functional analysis of bio-resources

Group	Assay	Aim
Basic assay	DPPH assay	Antioxidant
	Total polyphenol assay	Antioxidant
	MTT assay	Anti-cancer Cytotoxicity
	LDH assay	Anti-cancer Cytotoxicity
	Intracellular ROS assay	Antioxidant
	Intracellular F-actin assay	Differentiation
	Intracellular ATP assay	Energy metabolism
Neuronal assay	Acetylcholinesterase assay	Differentiation
	Neurite outgrowth assay	Differentiation
	Neuroprotection assay	Neuroprotection
Intestinal assay	TER (transepithelial electrical resistance) assay	Tight junctional regulation
	α-Glucosidase assay	Anti-diabetes
	Cholesterol assay	Anti-hyperlipidemia
	Epithelium permeability assay	Intestinal permeability
Immunity assay	β-hexosaminidase assay	Anti-allergy
	Histamin assay	Anti-allergy
	Intracellular Ca ion assay	Anti-allergy
	Immunoactivity assay	Immunoactivity
Anti-cancer assay	Leukemia differentiation assay	Anti-cancer
	Cancer migration assay	Anti-cancer
Lifestyle-related disease assay	Adipogenesis assay	Insulin resistance
	Adiponectin assay	Insulin resistance
	ACE Ⅱ assay	Hypertension
	Alkaline phosphatase activity assay	Osteogenesis
	Stress recovery assay	Anti-stress
Skin assay	Melanogenesis assay	Melanogenesis regulation
	Dermal papilla growth assay	Hair growth
	Epithelium permeability assay	Skin permeability

Table 2. The mechanism analysis methods list for functional molecular.

Group	Method	Aim
Mechanism analysis	Western blotting	Protein expression
	Real time-PCR	mRNA expression
	Micro array	Gene expressions
	Cell cycle	Cell division
	Proteomics	Protein expressions
	DNA fragmentation test	Apoptosis
	Immunostaining	Protein localization
	ELISA	Protein expression

Table 3. The species number of annual research (2010-2011) on each bio-resources.

	2010	2011
Olive	10	25
Halophyte	12	12
Aroma plant	13	13

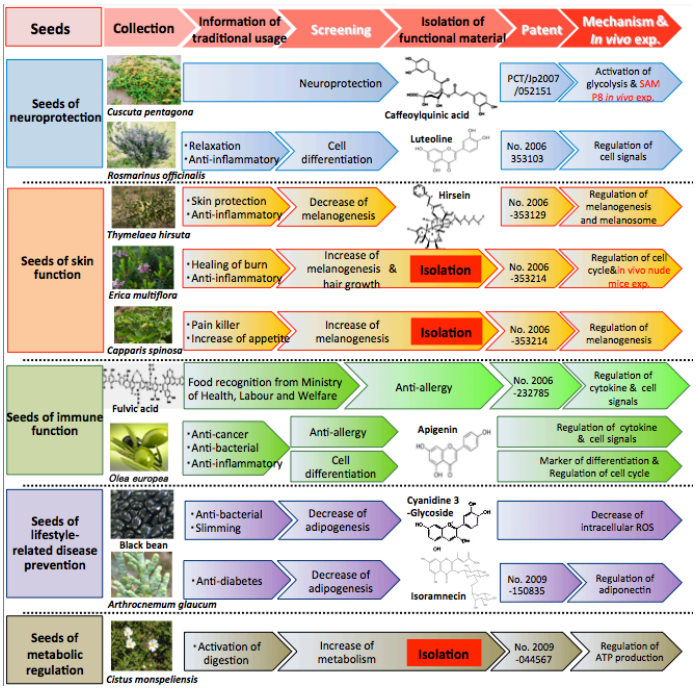
research for the application of functional food, medicine and cosmetic. Moreover, we hope that our research can contribute to the development of bio-resources region.

The obtained results of SATREPS project were used as contents of the SATREPS database. About new finding, we

Table 4. The results of bio-resources (Olive, Halophyte, Aroma plant) in 2010.

Input	Contents	Output
Olive	Anti-allergy effect Anti-cancer effect	Database
Halophyte	Anti-cancer effect Anti-fat effect Activation of energy metabolism	Database Patent Paper
Aroma plant	Anti-stress effect Melamogenesis regulation effect	Database Paper

Fig. 2. The road map of SATREPS functional analysis group work.



will apply to patent, then present by international journal and conference.

Figure 2 shows that the road map of SATREPS functional group work. To develop the possibilities of these seeds, we have planning to do the isolation of functional materials and the clinical test.

4. Conclusion

We investigated anti-oxidative activities from local bio-resources that have been used as traditional medicines. Plants that grow on arid land possess functions that protect them from the stress of harsh habitat, and it is thought that they contain unique functional components that play such roles. From our SATREPS project, we found that olives, halophytes and aroma plants that grow in Tunisia have anti-oxidant activity, anti-cancer activity, anti-allergic activity, anti-fat activity, melanogenesis regulation activity, energy metabolism activity and anti-stress activity, etc.

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