Study on Morphological, Preliminary Phytochemical Test, Nutritional Values and Antimicrobial Activities of Fruits of *Docynia indica* (Wall.) Decne.

Lai Win Aung, Khin Ei Chaw, Aye Aye Aung

Abstract

Docynia indica (Wall.) Decne is tree semievergreen or deciduous, belongs to the family Rosaceae in the order Rosales. It is locally known as Pin-Sein Thee. It was collected from Loi Mwe Hill, Kyaing Tong Township, East of Shan State. The morphological characters of vegetative and reproductive parts of plants were identified and classified by using with the help of available literatures. Then, the fruits were dried powdered and kept in airtight container to the sensory characters of powdered drug. Phytochemical analysis of powdered fruits indicated that the presence of alkaloids, saponins, reducing sugar, carbohydrates, glycosides, flavonoids, tannins, phenolic compounds, terpenoids and steroids. The nutritional values of fresh fruits such as protein, fat, fiber, carbohydrate, citric acid, vitamin C and energy value was conducted at Department of Research and Innovation Analysis Department. By using paper disc diffusion method, antimicrobial activities of various crude extracts were carried out with six test organisms. The ethanolic and methanolic extracts are exhibited the highest against on different pathogenic microorganisms. Therefore, it was observed that *Docynia indica* (Wall.) Decne is endowed with antimicrobial activities, nutritional value and bioactive constituents that can be provided useful in traditional medicine.

***Keywords*:** Morphology, Phytochemical, Nutritional value, Antimicrobial activity

Introduction

Plants are well known as a major source of modern medicines. Medicinal plants are rich source of natural products. It is documented that 80% of the world population had faith in traditional medicines particularly plant drug for their primary health care. Medicinal plants are an essential part of the traditional health care systems. There are more than 8000 plants species in South Asia with well known medicinal uses. *Docynia indica* (Wall.) Decne. can be found in India, Myanmar and Southern provinces of China and Vietnam. It is traditionally used in the form of syrup, alcohol, wine and vinegar. It has grown over the last few years, due to consumers, belief in its positive effects on human health. It has traditionally been used as a supplemental remedy to stimulate digestion and appetite and treating bloating and heartburn. Fruits are also used to treat different diseases through traditional methods (Shende, 2016 and Khrunomo and Ranjan, 2018).

The genus Docynia is a tree member of subfamily Maloideae, family Rosaceae, a large family comprising about 115 genera and 3200 species (Lawrence, 1963). *Docynia indica* (Wall.) Decne. is trees, semievergreen or deciduous, 4-8m tall. Leaves are simple and alternate. Flowers are white, hypanthium campanulate. There are numerous stamens. Fruits arepome, globose, ovoid, yellowish green with orange spot, persistent or incurved sepals. Seeds are black, glabrous.

Locals use the juice of the ripped fruit to treat conjunctivitis by putting it in the eye of the diseased animals. They also use this juice, about 6 teaspoons consumption of its fruit can also help reduce the risks of colon cancer and can prevent the growth of polyps. The major disease for the prevention and treatment of which nutraceuticals have been associated are heart diseases, cancer, hypertension, and diabetes (Sarla and Chandra *et al*., 2011). Its fruits are good source of pectin, help in maintaining desirable acid balance in the body (Petkou and Vasilakakis, 2002). This plant is used as medicinal for chickenpox, lesions, diarrhea, dysentry, anticancer,

diuretic, glands in the neck, diabetes, skin diseases, cough, fever (Kirtikar and Basu, 1975 and Backer, 1965).

Docynia have been known to have various physiological activities and have various useful polyphenol phytoconstituents, chlorogenic acids, arbutin etc. These polyphenols have already been reported for their antioxidant potential, antimutagenic and anti-carcinogenic effects. The nutritive contents of fruits are about sugars, protein, ash, pectin. It also contains a low content of Vitamin C (Hislolm, 1911). The fruit contains moisture total soluble solids, acidity, total sugar, reducing sugars tannins, vitamin (Parma and Kaushal, 1982). Fruits being a major forest product, supplement human diet as they provide essential vitamins, minerals and fiber required for maintaining health (Khruomo and Rajan 2018).

Phytochemical screening of *Docynia indica* (Wall.) Decne. showed that alkaloids, flavonoids, tannin, saponin, phenol and steroids (Khrunomo *et al*., 2018, Shende *et al*. 2016, and AFLI Techanical Report 2014 .

Methanolic and ethanolic extracts of fruits showed activity highest against on *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. Medicinal plants have very high potential as antimicrobial drugs for treating various human diseases. Although a number of plants have screened, the search for antimicrobial substances from plants is continued as better and safer drug to combat bacterial and fungal infections are still needed (Buwa and Staden, 2006 and Sharma, 2013).

Materials and Methods

(i) Morphological characters of *Docynia indica* (Wall.) Decne

The selected plants *Docynia indica* (Wall.) Decne was collected from Loi Mwe Hill, Kyaing Tong Township, East of Shan State. It is locally known as Pin-Sein Thee. The morphology study of the plant was undertaken with the help of available literatures, Lawrence (1964), Kirtikar and Basu, (1975), Ceylon (1995) and Flora of Hong Kong (2008). The collected plant parts were recorded with photo images.

(ii) Phytochemical constituents of fruits of *Docynia indica* (Wall.) Decne

Phytochemical examination was carried out in Myanmar Pharmaceutical Industrial Enterprise, Research Department, Insein Township, Yangon, according to the methods of Central Council for Research in Unani Medicine (1987), Marini Bettolo *et al.*, (1981). The fruits were dried at room temperature for one week. When samples were completely dried, pulverized by grinding machine and to get powdered and stored in airtight containers.

(iii) Nutritional values of fresh fruits of *Docynia indica* (Wall.) Decne

Nutritional values of energy, carbohydrate, fiber, protein, fat, citric acid and vitamin C were investigated according to the method of Association of Official Agricultural Chemists (A.O.A.C) Horwitz, 1997. The experiments were carried out at Department of Research and Innovation Analysis Department at the Union of Myanmar Ministry of Education.

(iv) Microorganism and Antimicrobial activity determination use for the tests

Preparation of nutrient agar and procedures for antimicrobial activities were carried out by using Cruickshank (1968) and Atlas (1993) methods. Nutrient Agar Medium was prepared by Agar - 25 g, Peptone - 0.5 g, Yeast - 0.2 g, Distilled water - 100 L and pH - 7.0 ±. The antimicrobial activity of the 50 ml of acetone, ethanol,

ethyl acetate, methanol, pet-ether and water extract was determined by individual testing on *Bacillus subtilis*, *Candida albican*, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Salmonella typhi*.

After autoclaving nutrient agar 20 - 25 ml of the medium was poured into petri-dishes and made plating by using 0.1 to 0.2 ml to test organisms. These plates were allowed to set for 2 - 3 hours. And then, 10 mm paper disc were absorbed about 0.5 - 0.8 ml of extract. 10 mm paper disc was introduced into agar plate and incubated at room temperature during one day. The clear zones appeared around. The paper disc indicates the presence of antimicrobial activity. The extent of antimicrobial activity shown by clear zone was measured with the help of clipper.

Results

(i) Morphological characters of *Docynia indica* (Wall.) Decne

Docynia indica (Wall.) Decne is perennial, tree semievergreen or deciduous. The leaves are alternate and simple. Inflorescences are axillary and cymes. Flowers are white, hypanthium campanulate, base shortly clawed. The stamens are numerous (20-30), unequal filament white, anther yellow. Carpels five, axile placentation, one to three ovules in each locule. Fruits are pome, yellowish green with orange spot, calyx persistant. Seeds are four to five, black. Flowering period – January to April and Fruiting period – March to August

(ii) Preliminary Phytochemical constituents of fruits of *Docynia indica* (Wall.) Decne

In preliminary phytochemical investigation, the presence of alkaloids, glycosides, tannins, starch, steroids and terpenoids were observed in fruits. Tannins, glycosides, terpenoids and phenolic compounds were more observed.

(iii) Nutritional values of fresh fruits of *Docynia indica* (Wall.) Decne

Nutritional evaluation from the fresh fruits of *Docynia indica* (Wall.) Decne was investigated in energy 31.84 Kcal / 100 g, carbohydrate 7.96 %, fibre 1.59 %**,** Vitamin C 0.005 %, Citric acid 1.82 %, fat - ND and protein - ND at Department Research and Innovation Analysis Department. Energy was greater than the other components.

(iv) Antimicrobial activity of different solvent extracts of fruits of *Docynia indica* (Wall.) Decne

In this experiment, the best antimicrobial activity effect of fruits extracts were found at ethanolic and methanolic extracts against *Bacillus subtilis*, *Candida albican*, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Salmonella typhi.* Methanolic and ethanolic extracts the highest indicated that antimicrobial activity against on *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi.*

Figure 1. Morphological characters of *Docynia indica* (Wall.) Decne

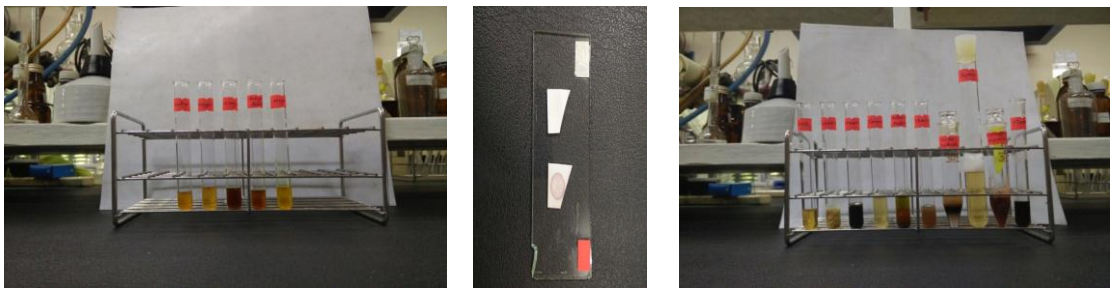
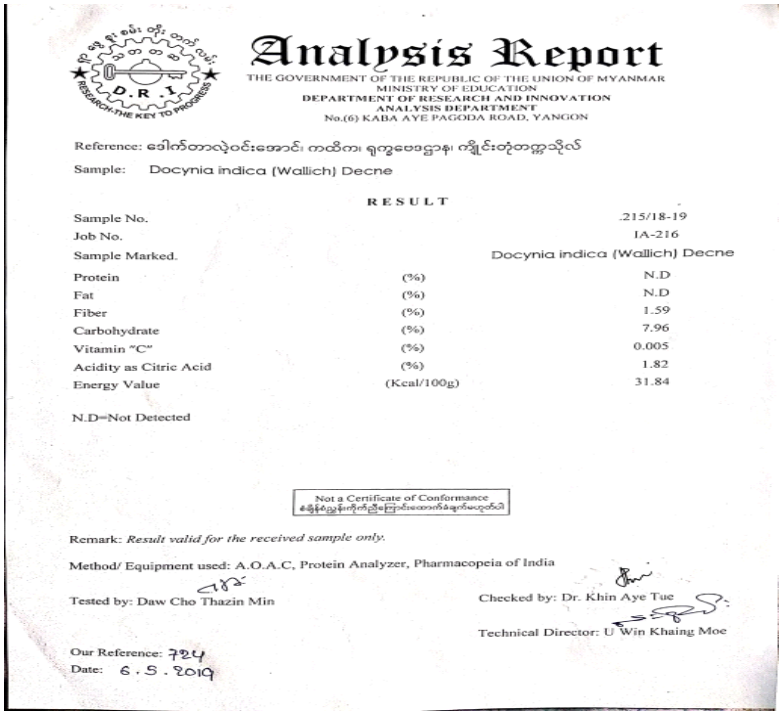


Figure 2. Preliminary phytochemical test of fruits of *Docynia indica* (Wall.) Decne.

Table 1. Preliminary phytochemical test of fruits of *Docynia indica* (Wall.) Decne.

	Test	Type of extract	Test Reagents	Observation	Fruits
1.	Alkaloids	HCl	(1) Mayer's Reagent	White ppt.	-
		HCl	(2) Wagner's Reagent	Yellow ppt.	+
		HCl	(3) Dragendorff's reagent	Orange ppt.	-
2.	Glycosides	H_2O	10% lead acetate solution	White ppt.	+
3	Reducing Sugars	H_2O	Benedict's solution	Brown ppt.	+
4.	Saponins	H_2O	Distilled water	Frothing	+
5.	Carbohydrates	H_2O	10%a-naphthol + H_2SO_4	Brown	+
	-Amino acids	H_2O	Ninhydrin Reagent	Pink spot	+
7.	Phenolic compounds	H_2O	Ferric chloride	Brown	+
8.	Flavonoids	EtOH	FICL/ Mg	Brown and Yellow	+
9.	Tannins	H_2O	Ferric chloride	Deep Blue	+
10.	Starch	H_2O	Iodine	Blue Black	+
11.	Terpenoids& Steriods	Pet - ether	Acetic anhydride + Conc. H_2SO_4	Pale and White	+



Analysis Report

THE GOVERNMENT OF THE REPUBLIC OF THE UNION OF MYANMAR
MINISTRY OF EDUCATION
DEPARTMENT OF RESEARCH AND INNOVATION
ANALYSIS DEPARTMENT
No.(6) KABA AYE PAGODA ROAD, YANGON

Reference: ဒေါ်တာလဲ့ဝင်းအောင်၊ ကထိက၊ ရုက္ခဗေဒဌာန၊ ကျိုင်းတုံတက္ကသိုလ်

Sample: Docynia indica (Wallich) Decne

RESULT

Sample No.		.215/18-19
Job No.		IA-216
Sample Marked.		Docynia indica (Wallich) Decne
Protein	(%)	N.D
Fat	(%)	N.D
Fiber	(%)	1.59
Carbohydrate	(%)	7.96
Vitamin "C"	(%)	0.005
Acidity as Citric Acid	(%)	1.82
Energy Value	(Kcal/100g)	31.84

N.D=Not Detected

Not a Certificate of Conformance

Remark: *Result valid for the received sample only.*

Method/ Equipment used: A.O.A.C, Protein Analyzer, Pharmacopeia of India

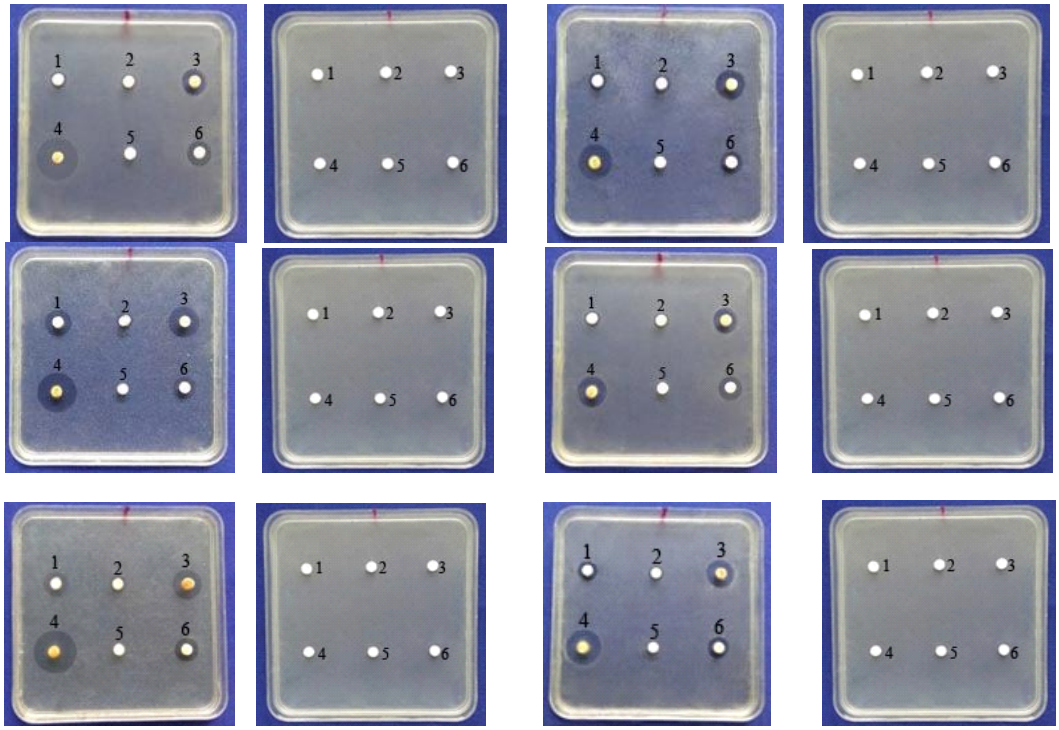
Tested by: Daw Cho Thazin Min

Checked by: Dr. Khin Aye Tue

Technical Director: U Win Khaing Moe

Our Reference: 724
Date: 6.5.2019

Figure 3. Nutritional values of fresh fruits of *Docynia indica* (Wall.) Decne



Paper disc size= 6mm

Figure 4. Antimicrobial activity of different solvent extracts of fruits of *Docynia indica* (Wall.) Decne.

Table 2. Antimicrobial activity of different solvent extracts of fruits of *Docynia indica* (Wall.) Decne.

No	Test Organisms	Acetone	EtOAc	EtOH	MeOH	PE	H_2O
1	*Bacillus subtilis*	8	8	14	18	-	10
2	*Candida albicans*	8	8	16	18	-	10
3	*Escherichia coli*	16	8	16	22	-	10
4	*Pseudomonas fluorescens*	8	8	14	16	-	10
5	*Staphylococcus aureus*	10	8	16	20	-	10
6	*Salmonella typhi*	12	8	18	24	-	10

Table 3. Sensory characters of the fruits of *Docynia indica* (Wall.) Decne.

	
Sample **Sensory characters**	Powdered of fruits
Colour	Reddish-brown
Odour	Aromatic
Taste	Sour sweet taste
Texture	Granular

Discussion and Conclusion

The present investigation involved the morphology study of vegetative and reproductive parts of *Docynia indica* (Wall.) Decne. In this study, *Docynia indica* (Wall.) Decne is perennial, trees. The leaves are alternate and simple. Inflorescences are axillary and cymes. Flowers are white, hypanthium campanulate. The stamens are numerous (20-30) unequal filament white, anther yellow. Capels five, axile placentation, two to three ovules in each locule. Fruits are pome, yellowish green with orange spot, persistent or incurved sepals. Seeds are black, glabrous. These characters were agreed with Lawrence (1964), Ceylon (1995), Flora of Hong Kong (2008) and Kirtikar and Basu (1975).

The preliminary phytochemical test was carried out, *Docynia indica* (Wall.) Decne showed that secondary metabolites as glycosides, alkaloids, flavonoids, saponins, tannins, reducing sugars, phenolic compounds, carbohydrates, steroids and terpenoids. These phytochemical constituents of fruits of *Docynia indica* (Wall.) Decne were agreed with those mentioned by AFLI Techanical Report (2014), Shende *et al*., (2016) Khrunomo *et al*., (2018). Alkaloids, flavonoids and terpenoids are the main secondary metabolites that exhibit many pharmacological and properties in living cells (Rhodes, 1994). Phenolic compounds in plants are essential part of human diet and antioxidant properties (Khan and Islam, 2011).

According to the result from Department of Research and Innovation Analysis Department in the government of the Republic of the Union of Myanmar, the nutritional values of fresh fruits contained energy 31.84% Kcal/100g, carbohydrate 7.96%, fiber 1.59%, protein -ND and fat – ND, Vitamin C 0.005% and Citric acid 1.84%. But Stephen and Cuizhi *et al*., (2018) and Hanoi (2014) stated that *Docynia indica* (Wall.) Decne of fruits contain energy 31.44 Kcal, carbohydrates 6.90%, protein 0.42%, fat 0.2%, fiber 1.6 % and Vitamin C 2.3 %. Fruits being a major forest product, supplement human diet as they provide essential vitamins, minerals and fiber required for maintaining health (Kuman, 2008). Living organisms require available energy to stay a live; humans get such energy from food. Daily food intake of a normal adult: 6-8 MJ. Many carbohydrates and fats processing involved for energy production. There are six main classes of nutrients that the body needs: carbohydrates, proteins, fats, vitamins, minerals and water. It is important to consume these six nutrients on a daily basis to build and maintain healthy bodily function (Smith and Crosbie, 1998). Fruits being a major forest product, supplement human diet as they provide essential vitamins, minerals and fiber required for maintaining health (Kuman, 2008).

In this experiment, methonolic and ethanolic extracts showed that the highest activity against on *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*. The present results were agreed with Sharma (2013). He reported that, *Docynia indica* (Wall.) Decne of fruits extracts which exhibited significantly higher animicrobial activity. Methanolic extracts of fruits showed activity against on *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi.*

In conclusion, the selected plant is resistant to some strains of clinical microorganisms such as bacteria and fungi. Thus, *Docynia indica* (Wall.) Decne could be used for preparation of effective antimicrobial source. Plant can be used as herbal medicine due to result of the enrichment of bioactive, antimicrobial activity and nutritional value. The above data would be helpful in further study of the research and possible development of new drugs for the prevention and treatment of infection diseases.

Furthermore, the pharmacological actives of *Docynia indica* (Wall.) Decne should be undertaken.

Acknowledgements

We would like to express our deep gratitude to Dr Myat Myat Moe, Professor, Head of Botany Department, Dagon University, for allowing us to use the Departmental facilities and invaluable advices.

References

Atlas, R. M. (1993). **Handbook of Microbiolocal Media** CRC Press, London.

Backer, C.A. and R.C. Bakhuizen-Van Den Brink, Jr. (1965). **Flora of Java** (Vol.II) N.V.P Noordhoff, Groningen, The Netherland.

B.C. Sharma (2013) **In vitro antibacterial activity of certain folk medicinal plants from Darjieeling Himalayas used to treat microbial infections**, Postgraduate Department of Botany, Dajeeling Govt. College, India.

Central Council of Research in Unani Medicine, (1987). **Physiochemical standards of unani formulation**. Ministry of Health and Family Welfare, New Delhi, India.

Cruickshank, R., J.P. Guguid and R.H.A., Swain (1968). **Medical Microbiology** (11th ed). The English Language Book society and F. and S. Livingstone Ltd., London.

Dassanayake, (1995). **Flora of Ceylon** (Vol.IX), Published Amerid Publishing Co.Rvt.Ltd. New Delhi.

Horwitz, W. (1997). **Official methods of analysis of association of official analytical chemist** (13th ed). Washington DC: Benjiamin Franklin Station.

Hundley, H.G. and U Chit Ko Ko, (1961)**. List of Trees, Shrubs, Herbs and Principal Climbers etc.** Yangon: Supplt., Govt. Printin and Italy.

Khruomo and Ranjan, 2018. **Indigenous Wild Edible Fruits**, Vol 6 (2) pg 405-413, Department of Botany, Nagaland University, Lumami 798627, Nagaland, India.

Kumari A, 2008. **Nutritional quality, functional properties and value addition of underutilized fruits of Himachal Pradesh**, PhD Thesis submitted to the Himachal Pradesh Krish, Vishvavidyalaya, Himachal Pradesh, India.

Kirtikar and Basu, (1975). **Indian Medicinal Plant** (Vol.III), Bishen Mahendra Pal Sigh, Dehradun, India.

Lawrence (1963). **Taxonomy of vascular plants**. The Macmillian Company, New York.

Qi-ming and WU Delin (SCBG), Hong Kong Herbarium (2008). **Flora of Hong Kong** (Vol III, Agriculture, Fisheries and Conservation Department, Hong Kong.

Parma and Kaushal,1982. *Docynia indica* (Wall.) P-78-80. In Wild Fruits, Kalyani Publishers, New Delhi, India.

Petkou and Vaasilakakis, 2002. **Arbutin oxidation by Pear**, peroxides. Plant Science, 162; pp 115-119.

Rhodes, M.J.C.(1994). **Physiological roles for secondary metabolites in plants**: some progress, many outstanding problems, Plant Mol. Biology, 24,1-20.

Saklani, 2011. Evaluation of nutritional profile medicinal value and qualitative estimation in parts of Docynia indica (Wall.)

Smith, C.1998. **The science of energy a cultural history**, Energy Physics in Victorian Britain. The University of Chicago Press.

Stephen and Cuizhi *et al*., 2014. **Study on Nutrient compositions of son tra fruits *Docynia indica* (Wall.),** Department of Pharmacognosy, Theory of Pharmacognosy Study. Hanoi University of Pharmacy.

Study on nutrient compositions of son tra fruits *Docynia indica* (wall.), 2014, AFLI Techanical Report No.12.