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Extract of Medicinal lapsi *Choerospondias axillaris* (Roxb.) exhibit antioxidant activities during *in vitro* studies

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Abstract
Lapsi *Choerospondias axillaris* (Roxb.) fruit has a soft whitish sour flesh and green to yellow skin used to make pickles, fruit tarts and spicy candy in Nepal. The present study evaluated the antioxidant activity of the fruit of lapsi Choerospondias *axillaris* (Roxb.) *In vitro* using two different solvents: aqueous and ethanol. The antioxidant properties of the lapsi fruit extracts was quantified by the DPPH radical (2, 2-diphenyl-1-picrylhydrazyl) and the results showed that lapsi fruit possess a strong antioxidant power with higher percentage of inhibition of DPPH radical recorded in ethanolic extracts (98%), followed by ascorbic acid (95%) and aqueous extract (91%). The results clearly showed that lapsi fruits contain exploitable and potent antioxidant molecules, and could be promoted as a prospective dietary supplements and or Nutraceutical for both human and animal use.

Keywords: DPPH, Choerospondias axillaris, phenolic, antioxidant and lapsi fruit.

Introduction
Lapsi, *Choerospondias axillaris* (Roxb.) is a popular fruit tree of Nepal and many other Asian countries [1]. This deciduous tree can grow up to 20 meters tall and has smaller purple-brown branches. Lapsi fruit is about 3 centimeters long with green-yellow skin, and is incredibly sour if eaten raw. Inside the fruit is a large seed that is segmented so that it looks like a star [2]. To prepare the fruit, the skin is peeled, sometimes the seeds are also removed, and the fruit is mixed with salt, spices, or sugar to make various aachars. Lapsi *achar* can be intensely sweet, salty, sugary or spicy as depending on how it is prepared. The lapsi fruit is rich in Vitamin C [3] and known for its antibacterial, antimicrobial and antioxidant properties [4], [3].

Fruits are important sources of minerals, fibers and vitamins, which provide essential nutrients for the human health. Increased consumption of fruit and vegetables significantly reduce the incidence of chronic diseases, such as cancer, cardiovascular diseases and other aging-related pathologies [5]. The wild underutilized edible fruits can also play an important role as food supplement. Fruits offer protection against free radicals that damage lipids, proteins, and nucleic acids. Polyphenols, carotenoids (provitamin A), vitamins C and E present in fruits have antioxidant and free radical scavenging activities and play a significant role in the prevention of many diseases [6]. The antioxidant activity of polyphenols is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, singlet and triplet oxygen, or decomposing peroxides [7].

Antioxidants are chemicals that block the activity of other chemicals known as free radicals [8]. Free radicals are highly reactive and have the potential to cause damage to cells [9] that may lead to cancer. Free radicals are formed naturally in the body, and antioxidants interact with and neutralize them when overproduced, thus preventing them from causing damage [10]. These antioxidants are called endogenous antioxidants enzyme. However, under stress or pathological condition the endogenous antioxidant may not be sufficient to quench the damaging effect of free radicals, therefore the body relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs [11]. These exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables, and grains are rich sources of dietary antioxidants [12]. Some dietary antioxidants are also available [13] as dietary supplements.

In recent times natural antioxidants are gaining considerable interest among nutritionists, food manufacturers, and consumers because of their perceived safety, potential therapeutic value, and long shelf life. Plant foods are known to protect against degenerative diseases and ageing due to their antioxidant activity (AOA) attributed to their high polyphenolic content (PC) [14]. Epidemiological studies have shown that high consumption of fruits and vegetables is associated with a lower incidence of cancer, heart disease, inflammation, arthritis, immune

related diseases, neurodegenerative diseases and diabetes, and antioxidant components, such as vitamin C, vitamin E and carotenoids, Plant polyphenols appear to play a vital role in decreasing the development of such diseases [15].Plants, including herbs and spices, have many phytochemicals which are a potential source of natural antioxidant, e.g., phenolic diterpenes, flavonoids, alkaloids, tannins and phenolic acids [16]. Plant products have been reported to promote various activities like anti-stress, growth promotion, appetite stimulation and immunostimulation in aquaculture practices [25]. This information encourages us to investigate the antioxidant potential of lapsi fruits *Choerospondias axillaris* (Roxb.) *in vitro* using DPPH (2, 2-diphenyl-1-picrylhydrazyl) method.

2. Materials and Methods

A. Collection and Authentication of Lapsi Fruits

Fruits of lapsi *Choerospondias axillaris* (Roxb.), collected from Kathmandu local market were identified by National Herbarium and Plant laboratory, Department of Plant Resources, Ministry of Forests and soil Conservation, Government of Nepal. After identification, the samples were immediately transported to the Laboratory of Fish Nutrition, Biochemistry and Physiology, Central Institute of Fisheries Education (CIFE), Mumbai, India. Aqueous and ethanol solvent were selected because they have been reported to be among the best solvents for the extraction of antioxidant compounds.

Scientific classification

Kingdom	:	Plantae
Order	:	Sapindales
Family	:	Anacardiaceae
Genus	:	*Choerospondias*
Species	:	*axillaris* (Roxb.)

B. Preparation of crude extract of lapsi fruits

The crude extracts of the pulp of lapsi fruits were prepared separately by using ethanol (80%) and distilled water as described below:

1) Ethanol extraction: The fruits sample were individually washed with tap water and spread over newspaper for air drying under shade. After drying, the fruits were powdered using warring blender. A known quantity (10 g) of lapsi powder was taken in a 250 ml conical flask and added with 100ml of ethanol (80%). The ethanol lapsi powdered mixtures was kept for 24 hrs on orbital shaker. After 24 hrs, the extract was filtered using muslin cloth to exclude the residue. The extraction was done two times and the filtrate pulled together was centrifuge at 10,000 rpm for 5 minutes and the supernatant was collected. Then the supernatant was concentrated using water bath at temperature of 70 °C. A greasy final material (crude extract of lapsi fruits) obtained was transferred to screw-cap bottles, labeled and stored under refrigerated (4 °C) condition until use.

2) Aqueous extraction: 10g of air dried powdered of lapsi fruits were placed in a 250 ml conical flask containing 100 ml distilled water and boiled for 30 minutes at a temperature of 75 ^{o}C. The extracts were prepared following the method described by Fawole *et al.*, (2013) [17].

3) Determination of antioxidant activity DPPH (2, 2-Diphenyl- 1-picrylhydrazyl) assay

The radical scavenging activity of the fruit extracts was tested against 2, 2- diphenyl l-1- picryl-hydrazyl (Sigma-Aldrich) radical following the method described by Williams *et. al.*, (1995) [18] with slight modifications. The fruit extract samples stock solution (100 mg/ml) was diluted to working concentrations of (10, 20, 40, 80, 160, 320 and 640) µg /ml, in ethanol and distilled water. Fifty microlitre (50 µl) of crude lapsi fruit extracts was placed in test tubes and 2ml of 0.06 mM DPPH solution in methanol was added. The test tubes were incubated in dark for 30 minutes, and the absorbance was read at 517 nm. A blank solution containing the same amount of ethanol and DPPH was prepared and measured. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. All the measurements were taken in triplicate and the mean values calculated. Ascorbic acid was used as a standard. The IC50 values were determined as the concentration of the test mixture that gave 50% reduction in absorbance from that of the control blank. The radical scavenging activity was calculated using the following formula:

$$DPPH scavenge(\%) = \frac{(AB - AA)}{AB} \times 100$$

(Where AB is the absorption of blank sample and AA is the absorption of tested crude plant extract solution)

C. Chemicals and Reagents

2, 2- Diphenyl l-2- picryl-hydrazyl (DPPH) radical and ascorbic acid, were procured from Sigma Chemical, Mumbai, India.

3. Statistical analysis

The data were statistically analyzed by using statistical package SPSS version 20 in which one way ANOVA and Duncans multiple range tests (DMRT) was used to determine the significant ($P < 0.05$) differences between the means when necessary at 5% probability level. The results expressed as means ± standard error (SE).

4. Results and Discussion

Choerospondias axillaris (Roxb.), a large, deciduous, and dioecious sub tropical fruit tree has been recognised as a potential agroforestry tree for income generation for subsistence farmers in Nepal. The tree, locally called Lapsi, produces fruits with high vitamin C content, which are consumed fresh, pickled or processed into a variety of sweet and sour fruit products locally called Titaura and Candy. It is a tasty food products that are marketed locally and have good potentials for exporting [19].

Natural antioxidants present in the plants scavenge harmful free radicals from our body when consumed in the diets. Free radicals are any species capable of independent existence that contains one or more unpaired electrons which react with other molecule, and involved in many pathological conditions [20] and they play very important role in human health and beneficial in combating against several diseases like

cardiovascular disorders, lung damage, inflammation etc. [21]. These free radicals are highly unstable and when over produced in the body, it can damage the cells and tissues and may involve in several diseases. Thus, there is a need for antioxidants of natural origin because they can protect the body from any free radicals related diseases [22].

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. The most common reactive oxygen species (ROS) include super-oxide (O_2) anion, hydrogen peroxide (H_2O_2), peroxyl (ROO) radicals, and reactive hydroxyl (OH) radicals. The nitrogen derived free radicals are nitric oxide (NO) and peroxynitrite anion (ONOO) [23]. Antioxidant compounds in food play a key role in health promotion. Scientific evidences suggest that antioxidants reduce risk of chronic diseases including cancer and heart disease. Various antioxidant assay methods have been used to monitor and compare the antioxidant activity of foods and herbal drugs [24].

Various methods [25] used to measure antioxidant activity of food products [26] can give varying results depending on the specific free radical being used as a reactant. These methods can be time consuming because they depend on the oxidation of a substrate which is influenced by temperature, pressure, matrix etc. and may not be practical when large numbers of samples are involved. Therefore, a rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of 2, 2- Diphenyl-1-picryl hydrazyl radical (DPPH). DPPH [27] is widely used to test the scavenging ability of compounds, and to evaluate antioxidant capacity of food products. A measure of total antioxidant capacity helps to understand the functional properties of food and food products [28]. Thus, different concentrations of the ethanolic and aqueous extracts of lapsi *Choerospondias axillaris* (Roxb.) ranging from 10 to 640 µg/ml were tested for their antioxidant capacity as measured by DPPH radical scavenging assay method. Higher radical scavenging effect was observed in both the ethanol and aqueous extracts of Lapsi fruits (Table 1). The solvent appears to be ideal for extracting antioxidant molecules from Lapsi powdered. Similar observation was made by Fawole *et al*. [29] after testing the radical scavenging power of different plants using aqueous and ethanol solvent. In the present study, the ethanolic extract scavenged more than 99% of DPPH radicals at 640 µg/ml concentration, while aqueous extract recorded 91%. Both the extracts are comparable to the ascorbic acid (95%) which served as control. The higher radical scavenging effect observed in the test sample may be attributed to the ability of the Lapsi fruits to donate its electron to free radicals in other to break the chain of reaction. It was also observed that the radical scavenging activity of ethanolic extracts of lapsi fruits was as strong as that of vitamin C compared to the water extracts, and the reason may be due to the Vitamin C present in the Lapsi fruits coupled with the polyphenol. Several previous studies [30], [31] have revealed that phenolic contents in plants are associated with antioxidant activities probably due to their redox properties that allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. The lowest IC50 value was found in ethanolic extracts compared to aqueous and ascorbic acid.

Table 1: DPPH scavenging activity of *C. axillaris* fruit.

Concentration (µg/ml)	% of inhibition		
	Aqueous Extract	Ethanol Extract	Vitamin C
640	91.20±0.22^{a}	99.84±0.55^{ab}	95.60±0.19^{a}
320	79.36±0.36^{ab}	87.49±0.72^{c}	78.14±0.39^{b}
160	68.43±0.29^{a}	74.35±0.64^{ab}	61.71±0.26^{bc}
80	51.21±0.84^{bc}	61.79±0.42^{b}	48.47±0.15^{ab}
40	31.18±0.71^{c}	35.49±0.19^{bc}	26.16±0.25^{a}
20	11.44±0.14^{a}	17.57±0.14^{bc}	11.57±0.14^{b}
10	5.76±0.44^{c}	8.43±0.19^{ab}	7.43±0.19^{b}
IC50 (µg)	195.12	141.63	205.27

Groups with different alphabetic superscripts differ significantly at P < 0.05 (ANOVA single factor with DMRT & SPSS-v20)
Values are mean (n = 3).

Aqueous and alcoholic extract of fruits has revealed the presence of most of the phytoconstituents [31] and moreover the presence of these phytochemical compounds in the ethanolic extract has showed maximum antibacterial activity in several studies [24, 28, 31, 20, 17].

5. Conclusion

On the basis of the results obtained in the present study, it is concluded that ethanolic extracts of *Choerospondias axillaris* fruits, which contains large amounts of phytoconstituents exhibits high scavenging and reducing power activities compared to aqueous extracts and control (ascorbic acid). These *in vitro* assays indicate that *C. axillaris* is a good source of natural antioxidant, which may be helpful in preventing oxidative stress *in vivo*. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the Lapsi fruit extracts. Furthermore, the *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

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