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TOTAL PHENOLIC, TOTAL FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITY OF A FEW INDIGENOUS FRUITS GROWN IN MANIPUR

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ABSTRACT
Fruits are excellent sources of natural antioxidants, flavonoid and phenolic compounds. Methanolic extracts and petroleum ether extracts of 3(three) wild fruits have been measured by DPPH (1, 1-diphenyl, 2-picrylhydrazyl radical) method for antioxidant activity, Folin-Ciocalteu method for phenolic content and $AlCl_3$ colorimetric assay for flavonoid contents respectively. The result indicates that high anti-oxidant, flavanoids and phenolic contents were observed in the methanolic extracts of the fruits as compared to the petroleum ether extracts. These results also confirmed that all the complex molecules like flavonols, polyphenols etc. are soluble in methanol as compared to petroleum ether.

INTRODUCTION

Manipur which lies within the Indo-Burmese mega biodiversity hotspot region in the north east India is a genetic treasure house of rich biological resources. This active center of speciation represents a zone of gene diversity for a variety of wild as well as domesticated plants and a secondary centre for several economically important medicinal and fruit plants. Fruits are known to contain a variety of different antioxidant compounds such as ascorbic acid, tocopherol, glutathione and carotenoids, which may all contribute to protection against oxidative damage (Bloknina *et al.,* 2003). Antioxidants, scavenge for free radical, consequently are very special group of nutritional supplements. Being a strong reducing agent, it helps to tie up free radicals and thus protect the body from their deleterious effects (Sumati *et al.,* 2003). This shows that fruits having good source of phenol and flavonols are a strong antioxidant. The free radicals have a strong tendency to impair the proper functioning of the immune system which leads to infection and degenerative diseases. Due to biochemical processes occurring in the body, it is normal for free radicals to be present in the body at all times however when the free radical posses chain reaction breaking properties. Among the numerous antioxidants available, flavonoid is naturally occurring phenolic compounds in plants. The antioxidantive effect of flavonoids had long been recognized. They are known to inhibit lipid peroxidation to scavenge free radicals and active oxygen to chelate iron ions and inactivate lipooxygenase (Tolmasoff *et al*., 1998). It has been recently shown that phenolic compounds from edible fruits are effective in-vitro antioxidants. Frankel *et al*. (1995); Fogliano *et al*. (1999) have reported that plant phenols in red wine exerted cardio protective effect. Keli *et al*. (1996) suggested an inverse relationship in the incidence of coronary heart disease and stroke in elderly men with dietary intake of flavonoids from tea, fruits and vegetables in human populations. Some reducing compounds or antioxidants such as ascorbic acid and glutathione are very efficient in controlling enzymatic browning. Thus, endogenous phenolic may also play a role in inhibiting fruit browning processes. Nutritional quality of fruit tissue is in part a function of carbohydrate metabolism, colour, pigment, and flavonoid, phenolic content and antioxidative capacity. Antioxidants provide chemical protection for biological systems against harmful effects of reaction or processes that cause excessive oxidation, protein and DNA damage and cell death Papas (1999), Arnao *et al*. (2011). Several studies have indicated that antioxidants prevent the onset of degenerative illness such as certain cancers, cardiovascular and neurodegenerative diseases, cataracts, oxidative stress dysfunction and aging (Schwartz, 1996; Papas, 1999; Deighton *et al.*, 2000; Arnao *et al.,* 2011). Recently, some studies have shown that a high intake of antioxidant food may decrease the risk of incidence of deadliest diseases (Ritaro *et al*., 2008). Most of developed countries like UK and Germany encourage and specifically advise increased consumption of

fruits (Smith and Somerset, 1993) as most of the nutritious values are easily available in them. Considering the importance of fruits in human health that are recognized in treatment of diabetes mellitus and hypertension Vaishali *et al*. (2003) and in various treatment of deleterious effects in human body (Mantene *et al*., 2003), cheap and rich source of vitamins, mineral and antioxidant like vitamin C may have a potential sources in these wild fruits found in Manipur. The paper deals with the antioxidant activity of plant contents.

MATERIALS AND METHODS

Collection and Identification

Different types of three (3) fruits were collected from local areas of Kakching Khunou, Manipur, North-East India. They are

(i) *Brucea javanica* Linn (Heining, local name)
(ii) *Elaeocarpus serratus* Linn (Chorphon, local name)
(iii) *Ficus palmata* Forsk (Heibam, local name)

These fruits are identified by Local taxonomist and herbariums are kept at Central Agricultural University, Imphal, India.

Preparation of extracts

Fresh fruits collected were washed and cleaned thoroughly in running tap water. Peels and seeds were separated manually. Peels were shredded into small pieces and dried at the room temperature separately. About 250g of the dried peels were extracted in a soxhlet apparatus successively with 4 times the amount of materials (V/W) of each solvent, petroleum ether & methanol each time until no more coloured matter was extracted. The low boiling, non-polar solvents (petroleum ether, methanol) and aqueous were taken to dryness in a rotavapour and freeze dried respectively.

Total phenolic assay

The total phenolic content of different extracts of fruits was determined by using Folin-Ciocalteu method Singleton and Rossi, (1965). An aliquot (1mL) of the extract (V/W) and standard solution of gallic acid (1, 2, 4, 5, 6, 8 and 10 mg) were taken in different test tubes and made upto the volume of 1mL with distilled water respectively. Then, 0.5mL of Merck Folin-Ciocalteu reagent (1:1 with distilled water) from amber coloured bottle and 2.5mL of 20% sodium carbonate solution were added to the test tubes sequentially. A reagent blank was prepared using distilled water. The reaction mixtures were mixed well and kept for incubation in dark at room temperature for 1h and the absorbance against the prepared reagent blank was determined at 725nm with a 100-bio Cary UV-visible spectrophotometer. The total phenol content was calculated and expressed as mg gallic acid equivalents (GAE)/100g weight. The samples were done twice.

Total flavonoid assay

The flavonoid content of each extracts was measured by the (Zhishen *et al.,* 1999) method of the $AlCl_3$ colorimentric assay. An aliquot (1mL) of extracts (V/W) or standard solution catechin (Sigma-Aldrich) (20, 40, 60, 80 and 100 mg/mL) was added to10mL volumetric flask containing 4mL of distilled water. To the flask was added 0.3 mL 10% $AlCl_3$. Again 2mL 1M NaOH was added and the total volume was made upto 10mL with distilled water and the solution were mixed well and the absorbance was taken against the prepared reagent blank at 510 nm. Total flavonoid contents of the whole plant were expressed as mg catechin equivalent (CE)/100g dried mass. Samples were analyzed thrice.

Anti-oxidant activity

The anti-oxidant properties of different extracts obtained consequently were measured by using 1, 1-diphenyl, 2-picrylhydrazyl radical (DPPH) according to (Okada and Okada, 1998). Of 1mL different concentration of extracts (2W/V) (10, 20, 40, 60, 80 and 100μL/mL) prepared in respective solvents were added to 4mL of a 0.01 mm (1.97165mg of DPPH in 50mL of ethanol in colored ember bottle) chloroform solution. The mixtures were shaken vigorously and left standing at room temperature in dark for 30 minutes. The absorbances of the resulting solutions were measured at 517nm after 30 minutes using 100 Bio Cary UV-visible spectrophotometer. The antioxidant activities (three replicates per treatment) were expressed as LC_{50} (mg/mL), the concentration required to cause a 50% DPPH inhibition. The anti-oxidant activities of the different fruits extracts were estimated by comparing with the standard ascorbic acid.

RESULTS AND DISCUSSION

From our finding, total phenolic content among the fruits were found to be maximum in methanolic extract of *Elaeocarpus serratus* Linn (chorphon) *i.e.* 400 mg/100g while minimum is *Ficus palmata* Forsk (Heibam) *i.e.* 160 mg/100g. Highest LC_{50} value is given by *Brucea javanica* Linn (92.30 mg/mL) followed by *Ficus palmata* Forsk (85.26mg/mL) and *Elaeocarpus serratus* Linn (64.10 mg/mL) .The LC_{50} value is the concentration of extract causing 50% inhibition of absorbance was calculated, since LC_{50} is a measure of inhibitory concentration, a lower LC_{50} value would reflect greater antioxidant activity of the sample. So *Elaeocarpus serratus* Linn has higher anti-oxidant activity than that of *Brucea javanical* Linn and *Ficus palmata* Forsk.

Table 1: Total flavonoid contents from petroleum ether extracts

Extracts name	Local name	Total flavonoidcontents (mg/100g)
Elaeocarpus serratus Linn	Chorphon	200
Brucea javanica Linn	Heining	550
Ficus palmata Forsk	Heibam	400

Table 2: Total flavonoid contents from methanolic extracts

Extracts name	Local name	Total flavonoidcontents (mg/100g)
Elaeocarpus serratus Linn	Chorphon	500
Brucea javanica Linn	Heining	610
Ficus palmata Forsk	Heibam	430

Table 3: Total phenolic contents from petroleum extracts are

Extracts name	Local name	Total phenolic contents (mg/100g)
Elaeocarpus serratus Linn	Chorphon	105
Brucea javanica Linn	Heining	170
Ficus palmata Forsk	Heibam	180

Table 4: Total phenolic content from methanolic extracts

Extracts name	Local name	Total phenolic contents (mg/100g)
Elaeocarpus serratus Linn	Chorphon	400
Brucea javanica Linn	Heining	110
Ficus palmata Forsk	Heibam	160

Table 5: Antioxidant activity from petroleum ether extracts

Extracts name	Local name	IC_{50} (mg/mL)
Elaeocarpus serratus Linn	Chorphon	26.10
Brucea javanica Linn	Heining	12.60
Ficus palmata Forsk	Heibam	18.70

Table 6: Antioxidant activity from methanolic extract

Extracts name	Local name	LC_{50} (mg/mL)
Elaeocarpu serratus Linn	Chorphon	64.10
Brucea javanica Linn	Heining	92.30
Ficus palmata Forsk	Heibam	85.26

In case of flavonoid content among the test samples, *Brucea javanica* Linn has higher value followed by that of *Elaeocarpu serratus* Linn and *Ficus palmata* Forsk. Details were shown in Table 1, 2, 3, 4, 5 and 6 respectively.

DISCUSSION

Flavonoids are the most common and widely distributed group of the plant phenolic compounds. Total flavonoid contents of petroleum ether and methanolic extracts for three samples varies. These are shown at below Table 1 and Table 2 respectively. According to the chemical composition *Brucea javanica* Linn contents a relatively highly percentage yield of flavonoid.

Phenolics are secondary metabolites that are ubiquitously present in fruits. Many of the phenolics have been shown to contain high levels of antioxidants activities Razali *et al*. (2008). Phenolic compounds contribute to the overall antioxidant activities plants mainly due to their redox properties. Generally, the mechanism of phenolic compounds for anti-oxidant activity are neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals Javanmardi *et al*. (2003). Total phenolic content of the extracts of fruits are demonstrated in Table 3 and Table 4. The petroleum ether and methanol extract of fruits have different phenolic contents.

From our finding, total phenolic content was highest in methanolic extract of *Elaeocarpus serratus* Linn (chorphon) *i.e.* 400 mg/100g while lowest is *Ficus palmata* Forsk (Heibam) *i.e.* 160 mg/100g. *Brucea javanical* Linn has 92.30 mg/mL (LC_{50} value) followed by *Ficus palmata* Forsk (85.26mg/mL) and *Elaeocarpus serratus* Linn (64.10 mg/mL) .The LC_{50} value for each fruit extract is defined as the concentration of extract causing 50% inhibition of absorbance was calculated, since LC_{50} is a measure of inhibitory concentration, a lower LC_{50} value would reflect greater antioxidant activity of the sample.So *Elaeocarpus serratus* Linn has higher anti-oxidant activity than that of *Brucea javanica* Linn and *Ficus palmata* Forsk. In case of flavonoid content among the test samples, *Brucea javanica* Linn has higher value followed by that of *Elaeocarpus serratus* Linn and *Ficus palmata* Forsk.

Primary antioxidant properties are generally measure by DPPH assay (expressed as LC_{50}). The DPPH assay measures the ability of the fruit extract to donate hydrogen to the DPPH radical resulting in bleaching of the DPPH solution (Lim Yau Yan *et al.,* 2006). The results of the antioxidant assay with petroleum ether and methanolic extract were reported in Table 5 and Table 6 respectively. From the present work it could be concluded that the solvent play a vital role in the extraction of the constituents.

As methanol and chloroform are highly polar among the solvents used therefore, they contain high yield of phenolic, flavonoid and antioxidant activity compounds respectively. The antioxidant activity could be co-related with the poly-phenolic components present in the extract. For a body to maintain anti-oxidant level, external supplementation is necessary for healthy living. In Manipur, a number of fruits based food, belonging to different families, possessing rich antioxidant properties is consumed by the people unaware of their nutritious significant and which perhaps may be the basis for low incidence of cancer. Thus, it can be concluded from the current investigation that exploring the source of natural antioxidant, phenolic and flavonoid content in this wild fruits grown in Manipur will help to re-introduce their use as food supplements and encourage their cultivation, conservation in home garden or by state government authority before these fruits are almost going extinct due to deforestation and urbanization. At the same time these fruits can be used as alternative source of natural antioxidant rather than synthetic antioxidant like Butylated hydroxyl toluene and Butylated hydroxyl anisole because of carcinogenicity (Mahdavi and Salunkhe, 1995).

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