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Ascorbic acid and tocopherol content of ten medicinal plant extracts of Manipur having anti-inflammatory properties

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Abstract
The present study on "Ascorbic acid and Tocopherol content of ten medicinal plant extracts of Manipur having anti-inflammatory properties" was undertaken to analyse the ascorbic acid and tocopherol content in ten medicinal plants of Manipur (*Cissus adnata, Debregeasia longifolia*, *Clerodendrum serratum, Polygonum barbatum*, *Colocasia gigantean*, *Allium hookeri, Houttuynia cordata, Oenanthe javanica*, *Allium odorum, Solanum xanthocarpum*). The ascorbic acid and tocopherol content of ten medicinal plants ranged from 15.23±0.05 to 48.08±0.04mg ascorbic acid equivalents/100g on dry weight, 1.5±0.11 to 6.09±0.08 mg α-tocopherol/100g on dry weight respectively. The presence of high concentration of vitamin C and vitamin E in ten medicinal plants might be responsible for their therapeutic effects and uses in the traditional system of medicine.

Keywords: Vitamin C, vitamin E, medicinal plants, inflammatory diseases

1. Introduction
Vitamins are organic substances necessary for metabolism. Human diet does not always contain the required amount of vitamins for the normal growth and maintenance of the body function and such cannot produce enough quantity for their body metabolism, so it can be obtained from fruits, vegetables and foods. Deficiency of vitamins can cause serious health diseases and sometimes, very small concentrations are required for maintenance of good health (Hussain *et al.,* 2006) [8]. Vitamin C plays an important role in prevention and treatment of oxidative stress, such as cancer, diabetes mellitus, asthma, cataract, HIV infection, (Evans *et al.*, 1996; Jaruga, 2002; Polidori, 2001) [6, 9, 12]. Vitamin C is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical. As a scavenger of ROS, ascorbate is effective against the superoxide radical anion, hydrogen peroxide, the hydroxyl radical, and singlet oxygen by accepting hydrogen atom (Weber *et al.,* 1996) [17] and the ascorbyl free radical can be converted back to reduced ascorbate by accepting another hydrogen atom. Vitamin C also scavenges reactive nitrogen oxide (RNS) species to prevent nitrosation of target molecule. It is necessary to form collagen, an important protein used to make skin, scar tissue, tendons, ligaments and blood vessels and also essential for the healing of wounds and for the repair and maintenance of cartilage, bones and teeth. Regular consumption of foods rich in ascorbic acid promotes iron absorption (Bamji *et al.,* 2003) [3]. Vitamin C is effective in protection against oxidative damage in tissues, and also suppresses the formation of carcinogens like nitrosamines (Gorton and Javis, 2000) [7].
Vitamin E is well recognised antioxidant because of its role in prevention of ROS generation thereby preventing onset of different diseases like coronary heart disease, arthritis, asthma, cancers, infectious diseases etc. (Tang *et al.,* 1997) [15]. It is also necessary for the proper functioning of the immune system, cell signalling, regulation of gene expression, antibody production, phagocytic and lymphocytic responses and exhibit resistance to viral and infectious diseases (Traber, 2006; Tang *et al.,* 1997; Allard *et al.,* 1998) [16, 15, 1]. Vitamin E functions primarily as an antioxidant by trapping peroxyl free radicals and it is considered as a master of antioxidant because it inhibits the bad cholesterol (LDL) which is believed to be the first step in the development of atherosclerosis So, it helps in preventing or delaying coronary heart disease by limiting the oxidation of LDL-cholesterol and prevents the formation of blood clots, which could lead to a heart attack. Tocopherols are basically the derivatives of

6-hydroxy chromane ring with three isoprenoid units which forms the side chain and its antioxidant property is due to the presence of chromane ring (Combs, 1992; Papas, 1999) [4, 11]. Medicinal plants are not only rich in minerals but also rich in vitamins which help in preventing many degenerative diseases. So, the following ten medicinal plants namely, *Cissus adnata* (Kongouyen), *Debregeasia longifolia* (U-Khajing), *Clerodendrum serratum* (Moirang khanam), *Polygonum barbatum* (Yelang), *Colocasia gigantea* (Yendem), *Allium hookeri* **(**Napakpi), *Houttuynia cordata* (Tuningkhok), *Oenanthe javanica* (Komprek), *Allium odorum* (Nakuppi), *Solanum xanthocarpum* (Leipung-kangkha) (shown in plate 1 and 2) were selected to carry out the present investigation.

2. Materials and methods

2.1 Collection of Plant Material

The required fresh plants/plant parts were collected on the advice of the traditional healers, from various places of Thoubal district (24°37′N and 93°30′E), Manipur, India and also from the local market of Manipur. The samples were collected during the month of December and January and also June and July, in the year 2013-2014.

2.2 Chemicals and reagents

Metaphosphoric acid (HO_3P), 2, 6 dichlorophenol indophenols ($C_{12}H_7NCI_2O_2$), Sodium bicarbonate ($NaHCO_3$), Ascorbic acid ($C_6H_8O_6$), Absolute alcohol (C_2H_6O), Xylene (C_8H_{10}), 2, 2′-dipyridyl ($C_{10}H_8N_2$), Ferric chloride ($FeCI_3$), α-Tocopherol ($C_{29}H_{50}O_2$), Sulphuric acid (H_2SO_4).

2.3 Preparation of sample

After collection the tender leaves or required plant parts were cleaned by removing the infested and diseased portion. The leaves were thoroughly washed under running water and finally in distilled water and shade dried till the leaves became very crisp. The dried plant material were then ground properly into fine powder in an electrical grinder and stored in an airtight container with identification labels. The ground plant species were stored in a refrigerator at 4°C. These powdered materials were used for further different chemical analysis.

Cissus adnata (Kongouyen)

Debregeasia longifolia (U-Khajing)

Clerodendrum serratum (Moirang khanam)

Polygonum barbatum (Yelang)

Colocasia gigantea (Yendem)

Plate 1: Selected medicinal plant samples

Allium hookeri **(**Napakpi)

Houttuynia cordata (Tuningkhok)

Oenanthe javanica (Komprek)

Allium odorum (Nakuppi)

Solanum xanthocarpum (Leipung-kangkha)

Plate 2: Selected medicinal plant samples

2.4 Estimation of ascorbic acid content

The ascorbic acid present was estimated by direct colorimetric determination (Ranganna, 1977). 5g of the sample were homogenized with 6% metaphosphoric acid and make up an aliquot of the macerate to 50ml. To dry cuvettes or test tubes pipetted out the requisite volumes of standard ascorbic acid solution-1, 2, 2.5, 3, 4 and 5 ml and make up the volume to 5 ml with the requisite amount of 2% meta-phosphoric acid. Added 10ml of dye with a rapid delivering pipette shake and took the reading within 15 to 20 sec. Set the instrument to 100% transmission using a blank consisting of 5ml of 2% meta-phosphoric acid solution and 10 of water and measured the red colour at 518 nm and plot absorbance against concentration. Placed 5 ml of the extract in a dry test tube and added 10 ml of dye and measure as in standard.

Calculation

mg of ascorbic acid per 100g of sample taken	=	Ascorbic acid content× volume made up×100 / ml of solution taken for estimation×1000×weight of the sample

2.5 Tocopherol content

The tocopherol content in the plant samples were estimated spectrophotometrically by the method reported by Ranganna (1997) [13]. The plant samples (0.5g) were homogenized in a 10 ml of 0.1N sulphuric acid and the volume was finally made up to 50 ml by adding 0.1N sulphuric acid slowly, without shaking and the contents were allowed to stand overnight. The contents of the flask were shaken vigorously on the next day and filtered through Whatman No.1 filter paper. Aliquots of the filtrate were used for the estimation of tocopherol. The plant extract, standard and water of 1.5ml were pipetted out into three centrifuge tubes namely test, standard and blank respectively. To all the tubes, 1.5ml each of ethanol and xylene were added, stoppered, mixed well and centrifuged. After centrifugation, the xylene layer was transferred into another tube, taking care not to include any ethanol or protein. To 1.0 ml of xylene layer, 1.0ml of 2, 2′-dipyridyl reagent was added, stoppered and mixed. This reaction mixture was taken in the spectrophotometric cuvettes and the extinctions of the test and the standard were read against the blank at 460nm. Then, in turn, beginning with the blank, 0.33ml of ferric chloride solution was added, mixed well and after exactly 15 minutes, the test and the standard were read against the blank at 520nm. The levels of tocopherol were calculated using the formula

$$\text{Tocopherol } (\mu g) = \frac{A_{520} - A_{460}}{StdA_{520}} \times 0.29 \times 15 \times \frac{\text{total volume of homogenate}}{\text{volume used} \times \text{weight of the tissue}}$$

2.6 Statistical analysis

The results of all experiments performed were expressed as Mean ± SD of three determinations.

3. Results and discussion

3.1 Ascorbic acid content

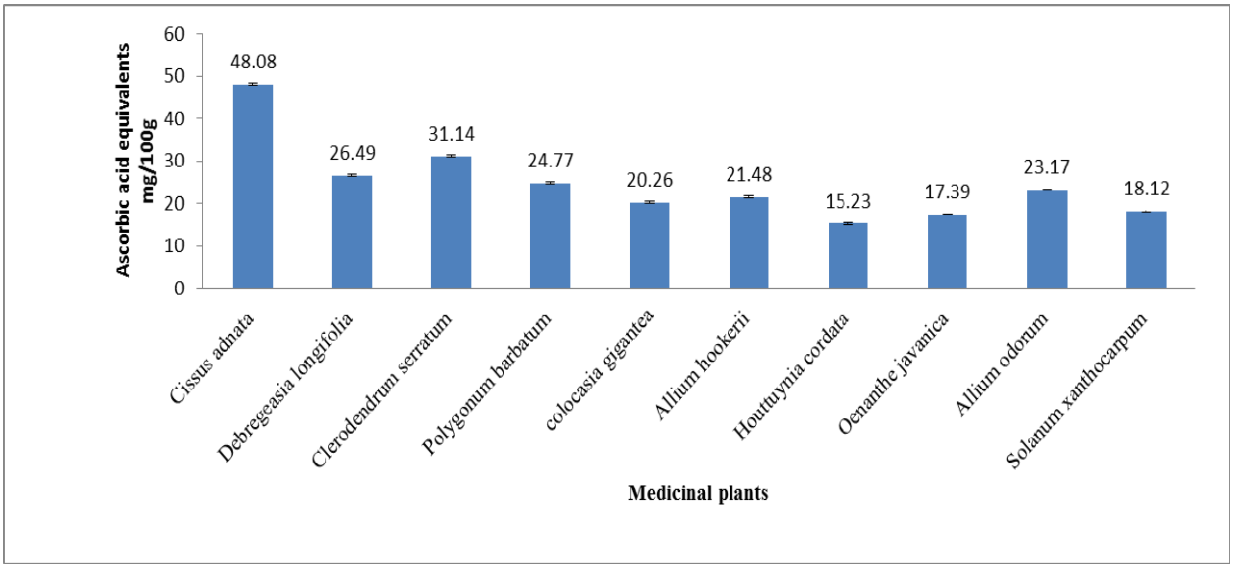

Fig 1: Ascorbic acid content in ten medicinal plant extracts

The ascorbic acid content of ten medicinal plants was analysed and it was expressed as ascorbic acid (AA) equivalents. It was observed that the ascorbic acid content of ten medicinal plants showed significant variation, ranging from 15.23±0.05 to 48.08±0.04mg ascorbic acid equivalents/100g. The highest ascorbic acid content was found in *Cissus adnata* (48.08±0.04mg/100g), followed by *Clerodendrum serratum* (31.14±0.08), *Debregeasia longifolia* (26.49±0.06), *Polygonum barbatum* (24.77±0.05), *Allium odorum* (23.17±0.05), *Allium hookeri* (21.48±0.05), *Colocasia gigantean* (20.26±0.06), *Solanum xanthocarpum* (18.12±0.005), *Oenanthe javanica* (17.39±0.06) and *Houttuynia cordata* (15.23±0.05). Vitamin C is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical. As a scavenger of ROS, ascorbate is effective against the superoxide radical anion, hydrogen peroxide, the hydroxyl radical, and singlet oxygen by accepting hydrogen atom (Weber *et al.*, 1996) [17] and the ascorbyl free radical can be converted back to reduced ascorbate by accepting another hydrogen atom. Vitamin C plays an important role in prevention and treatment of oxidative stress, such as cancer, diabetes mellitus, asthma, cataract, HIV infection, (Evans *et al.*, 1996; Jaruga, 2002; Polidori, 2001) [6, 9, 12]. Vitamin C also scavenges reactive nitrogen oxide (RNS) species to prevent nitrosation of target molecule. Vitamin C is necessary to form collagen, an important protein used to make skin, scar tissue, tendons, ligaments and blood vessels and also essential for the healing of wounds and for the repair and maintenance of cartilage, bones and teeth (Bamji *et al.*, 2003) [3]. Many studies have shown that vitamin C intake is inversely related to incidence of cancer, due to its protective effects on the lung, breast, pancreas, stomach, cervix, rectum and oral cavity (Simon *et al.*, 2001) [14]. Deficiency of vitamin C causes anaemia, scurvy, infections, bleeding gums, nutrient intake in relation to muscle degeneration, poor wound healing, atherosclerotic plaques and capillary haemorrhaging. It has been reported that diabetic individual have low levels of vitamin C in the plasma and in the white blood cells, which constitute our immune defence (Cunningham, 1991) [5]. The ten medicinal plants are rich in vitamin c which may prevent the related health problems associated with this vitamin deficiency.

3.2 Tocopherol content

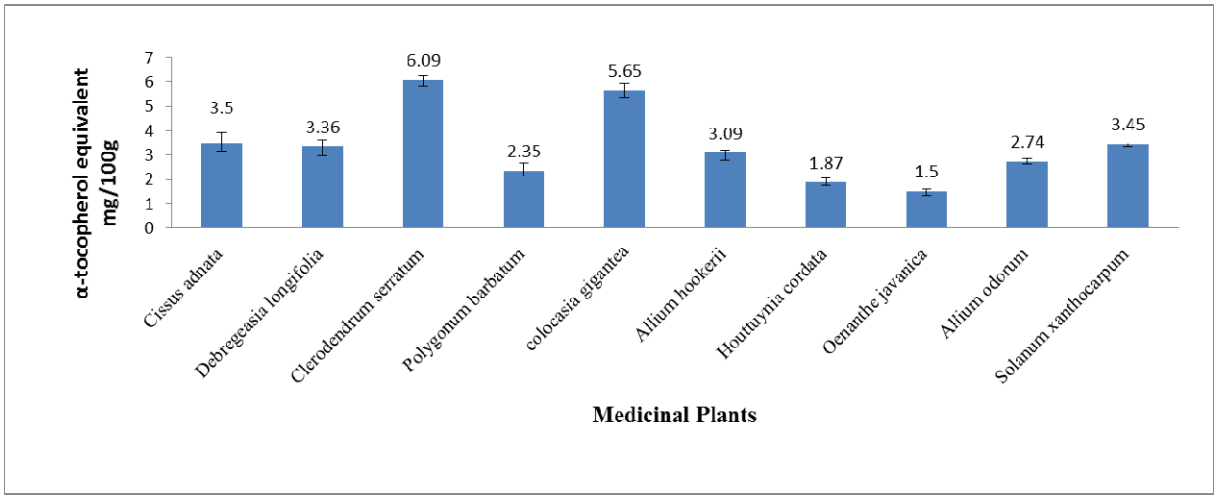

Fig. 2 Tocopherol content in ten medicinal plants extracts

The Tocopherol content of ten medicinal plants was analysed and it was expressed as α-tocopherol equivalents. From the Fig. 2, it was observed that the tocopherol content of ten medicinal plants ranged from 1.5±0.11 to 6.09±0.08 mg α-tocopherol/100g. The highest tocopherol content was found in *Clerodendrum serratum* (6.09±0.08 mg/100g), followed by *Colocasia gigantean* (5.65±0.06), *Cissus adnata* (3.50±0.04), *Solanum xanthocarpum* (3.45±0.12), *Debregeasia longifolia* (3.36±0.04), *Allium hookeri* (3.09±0.09), *Allium odorum* (2.74±0.05), *Polygonum barbatum* (2.35±0.08), *Houttuynia cordata* (1.87±0.13), and Oenanthe *javanica* (1.5±0.11). Vitamin E is well recognised antioxidant because of its role in prevention of ROS generation thereby preventing onset of different diseases like coronary heart disease, arthritis, asthma, cancers, infectious diseases etc. (Tang *et al.,* 1997) [15]. It is also necessary for the proper functioning of the immune system, cell signalling, regulation of gene expression, antibody production, phagocytic and lymphocytic responses and exhibit resistance to viral and infectious diseases (Tang *et al.,* 1997; Allard, 1998) [15, 1]. Tocopherols are basically the derivatives of 6-hydroxy chromane ring with three isoprenoid units which forms the side chain and its antioxidant property is due to the presence of chromane ring (Evans *et al.*, 2000) Vitamin E functions primarily as an antioxidant by trapping peroxyl free radicals and it is considered as a master of antioxidant because it inhibits the bad cholesterol (LDL) which is believed to be the first step in the development of atherosclerosis (Combs, 1992; Papas, 1999) [4, 11]. So, it helps in preventing or delaying coronary heart disease by limiting the oxidation of LDL-cholesterol and prevents the formation of blood clots, which could lead to a heart attack. Research has shown that Vitamin E delays or minimizes development of cataract and also has a protective effect against cataract formation induced by radiation, glucose, or galactose. Animal research has demonstrated the effectiveness of Vitamin E supplementation in inhibiting the elevation of free-radical concentrations related to arthritis (Yoshikawa *et al*., 1983) [18]. Vitamin E supplements decreases the risk of colon and prostate cancers and also reduce the risk of coronary disease by approximately 40 percent (Amens, 1998) [2]. Several studies have also reported that Vitamin E supplementation significantly showed more effective than placebo in relieving pain (Machtey and Ouaknine, 1978) [10]. Vitamin E content in selected ten medicinal plants may helps in preventing from various healths related issues.

4. Conclusion

The study showed that the studied ten medicinal plants contained high vitamin C and vitamin E constituents, which demonstrated that the ten medicinal plants might have good antioxidant activity and could be a good source of natural antioxidants. Consumption of these medicinal plants with high vitamin C and vitamin E content will result in improved health thereby reducing many diseases like cardiovascular diseases, diabetes, skin diseases, infertility, cancers etc. Further elaborate studies on the other vitamin content of the medicinal plants can be done to identify more functional properties that can be help in management of health and diseases.

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