Original Research article

Ethnopharmacognosy And Nutritional Composition Of *Stemona tuberosa* Lour. : A Potential Medicinal Plant From Arunachal Pradesh, India

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Abstract: The state Arunachal Pradesh is reported to harbor at least 500 species of medicinal and pharmacologically significant plant species. Among these *Stemona tuberosa* Lour. (Stemonaceae) plays a significant role in curing various diseases. The tuberous root is reported to have anti-larval property. The tuberous roots of the species is used for preservation of cultivated seed grains and also used as a mosquito repellent agent. The water extract of the root is taken orally to relieve abdominal pain, joint pain and stomach disorder. The root extract is also used for treatment of malaria. The present study deals with the nutritional constituents and ethnopharmacognosy of *Stemona tuberosa* Lour. The proximate composition like ash content (2.87%), moisture (6.82%), total sugar (20.32%), crude protein (10.06%), crude fat (0.67%) etc. were obtained. Minerals like Na (0.25%), K (0.54%), P (1.26%), N (1.61%), Fe (0.43%) were analyzed in the tubers thereby showing high nutritional value. The sugar content was also quantified. The preliminary phytochemical screening of the tuberous roots showed high amount of secondary metabolites.

Morphological and anatomical characters play a vital role in crude drug standardization and in pharmacognosy. The macroscopic and microscopic characters of the leaf, stem and roots were done for authentication of crude drug. Many granular and dark crystal structures indicated the presence of secondary metabolites. The ethnobotanical uses and the phytochemicals present in the roots of this plants need more elaborative studies and as such further work has already been initiated for its bioactivity study.

Key words: Ethnopharmacognosy, Nutritional value, *Stemona tuberosa* , Medicinal plant, Arunachal Pradesh

Introduction

The documentation of the medicinal plants has been a continuous process throughout the globe. Among the total recorded (2,97,000 – 5,10,000) plant species in the world (Schippmann *et al*., 2005), about (10-20)% is used as medicine in treatment of various diseases (Prajapati *et al*., 2003). As a mega biodiversity country, India harbors 17,500 plant species and among them 6000 species have been reported to have medicinal properties. More than 8000 angiosperm plant species have been reported from north-eastern region of India, of which 2500 have been reported for having medicinal properties (Trivedi, 2002). Thousands of tones of dried plant materials are sent every year to the developed countries for extraction of medicinal preparation (Adjanohoun, 1996). International export trade in medicinal plants has been dominated by China which exported 121 900 tons a year and India which exported 32 600 tons a year (Rajasekharan and

Ganeshan, 2002). More number of researchers and institutions need to be seriously involved in medicinal plants research and development, not only for the intellectual challenges involved but also the huge possible profits obtainable over a period of time (Latif, 1984; Osman, 1995; Rates, 2000).

The state of Arunachal Pradesh is very rich in floral and faunal heritage with its diverse geology, topography and climatic conditions. The state belongs to one of the top 12 mega biodiversity regions of the world, which is not only rich in terms of bio resources but also have rich ethnic diversity with more than 26 major and 110 sub tribes. Each and every tribe has their own traditional, medicinal and healthcare practices.

Stemona tuberosa Lour. first reported by William Roxburgh in 1795 from Andhra Pradesh, India and is a potential medicinal plant which is well known as wild asparagus described under the genus *Roxburghia* Roxb., as *Roxburghia gloriosoides* Roxb. The taxonomy of the species has been a controversial subject matter. It was earlier kept under the family Roxburghiaceae by Lindley (1832). However, later on several authors supported that the Stemonaceae is the appropriate family for *Stemona tuberosa* Lour. (Krause, 1930). Stemonaceae is represented with four genera, viz. *Pentastemona* Steenis., *Stichoneuron* Hook., *Croomia* Torrey. and *Stemona* Lour. having altogether 25 species (Hooker, 1892) distributed in various parts of the world. Only two species have been reported from India (Bora, 2003). However, *Stemona tuberosa* Lour. is distributed in some parts of Australia, Bangladesh, China, Combodia, India (Costal Andhra Pradesh, Northeastern states and North Tamil Nadu), Loas, Malayasia, Myanmar, Philipines, Thailand and Vietnam (Inthtachub, 2008).

Ethnobotanicaly, *Stemona tuberosa* Lour. has lots of medicinal uses. Mainly the tuberous roots are used as antitussive, anthelmintic and insecticide in Vietnam (Valkenburg, 2002), used in phthisis for coughs and chest complaints in Malaysia (Burkill, 1960), skin diseases in Myanmar (Chuakul, 2000), to treat scabies and kill head lice in Thailand (Valkenburg, 2002), to cure tuberculosis in lungs (i.e. pthisis) (Agarwal, 2005), soothes in human respiratory tract, antiseptic in India (Pattanaik, 2005), to manage respiratory diseases (bronchitis, pertussis and tuberculosis), prevent human and cattle parasites, agriculture pests and domestic insects in China and Japan (Cuzzupe, 2005) and the roots are used against mental disorder, worm, cough and jaundice in Bangladesh (Biswas, 2010).

The root of *Stemona* plants contains alkaloids. The tubers cotoluene, benzene, chloroform; m.p. 160°), which is mildly toxic. Tuberostemonine, stenine, oxotuberostemonine, stemonine, stemotinine and isostemotinine were identified in the root of *S. tuberosa* (Zhu, 1998). Two new alkaloids, named tuberostemoninol and stemoninoamide, were isolated from the roots of *S. tuberosa* (Lin *et. al.*, 1994). Some other alkaloids like neotuberostemonine and bisdehydroneotuberostemonine (Ye *et al.,* 1994), stenine (Ueo *et al.,* 1967) were also isolated from the roots.

However, there is scanty literature on the ethnobotany and the chemical constituent of *Stemona tuberosa* Lour. with no pharmacognostical and the nutritional analysis at all till date. Therefore, the present study deals with the ethnopharmacognostic and the nutritional studies of the tuberous roots of *Stemona tuberosa* Lour. from Arunachal Pradesh, India.

Materials and methods

The plant material was collected (Fig. 1 A & B) from Rajiv Gandhi University campus, Arunachal Pradesh. It was identified with the help of the flora of Arunachal Pradesh.

Fig. 1 . The plant *Stemona tuberosa* is a creeper (A) with the dried tuberous root (B).

The voucher specimen was deposited to Rajiv Gandhi University and herbarium was prepared following the methodology of Jain and Rao (1997). Important ethonobotanical utilization was recorded with the consultation of local people.

Pharmacognostic study

Macroscopy

Morphological studies like shape, size, apex, base of leaves, tuberous root and flowers were determined with the help of simple microscope.

Microscopy

Microscopic studies of leaf, stem, petiole and tuberous root were carried out by preparing a thin handmade section and stained with safranin. Quantitative microscopy i.e., number of stomata and stomatal index were determined by using fresh leaves of the plant (Kokate, 1994).

Plant material

Tuberous roots of the plant were collected and washed thoroughly in running water to remove soil and other dust particles. Roots were then cut into small pieces and dried in hot air oven at 35^{O}C for overnight. The dried samples were powdered and kept in moisture free container for further chemical analysis.

Preparation of plant materials

Powdered tuberous roots samples were soaked in different solvents i.e., acetone, chloroform, ethanol and water with occasionally shaking at room temperature for 48 hours. Samples were then filtered and kept for evaporation to concentrate the extract following slow evaporation method (Trease and Evans, 1989).

Phytochemical screening

Condensed extracts were used for preliminary screening of secondary metabolites such as alkaloids (Wagner, Hager and Mayer's test), carbohydrates and glycosides (Fehling, Benedict and Molisch's test), phenols and tannin (Lead acetate and $FeCl_3$ test), saponin (Foam and Haemolysis tests), steroid (Salkowski test), fixed oils and fats (Spot test), flavonoid (Lead acetate test) and proteins (Biurret test) by following standard procedures (Trease and Evans, 1989).

Proximate analysis

The proximate analysis of the samples i.e. moisture, crude fat, fiber, protein and ash were determined using different protocols from the manual i.e. methods in food analysis (Schanderl, 1970). All values are presented as average of triplicate analysis.

Mineral analysis

The mineral elements were determined in the solutions obtained above-Na and K by flame photometry, Model 405 (Corning, Halstead Essex, UK) using NaCl and KCl to prepare standards. Minerals were analyzed using the solutions obtained by dry ashing the samples at 55^{O} C and dissolving it in 10 % HCl (25 ml) and 5 % lanthanum chloride (2 ml), boiling, filtering and making up to standard volume with deionized water. Phosphorus was determined colorimetrically using a Spectronic 20 (Gallenkamp, London, UK) instrument, with KH_2PO_4 as a standard. All other elements (Ca, Mg, Zn, Fe, Mn, Cu and Cr) were determined by atomic absorption spectrophotometry, Model 403 (Perkin-Elmer, Norwalk, Connecticut, USA).

Results

Ethnobotanical utilization

Tuberous root extract of *Stemona tuberosa* is used as fish poison, mosquito repellant as well as for preservation of cultivated seed grains by certain communities of Arunachal Pradesh. An extract of the tuberous root is taken orally to relieve abdominal pain and stomach trouble. The root extract is also used for the relive from joint pain and malaria.

Macroscopic characters

Description of the plant

The species is herbaceous in nature, grows in moist shady places between 500-1500 m altitudes. It is categorized as least concern (LC) (Singh *et. al.*, 2012). It grows up to (3-5) m long, stems woody near to the base, smooth and glabrous. Roots tuberous, fleshy (10-30) x (2-3) cm^2 in size, creamy white in colour, spindle shaped. Leaves are generally opposite or whorled, broadly ovate or ovate lanceolate, acuminate at

apex, cordate at base, (10-25) x (5-15) cm^2, shining margin slightly undulate, lateral nerves 7-13, petioles (6 - 10) cm long. Inflorescences are axillary, racemose 1-many flowered, perianth subequal, lanceolate, (3.5-7.8) x (0.7-1.2) cm^2, greenish with purplish veins, bracts (0.5-2.3) cm long. Pedicels (0.5-2) cm long, tepals 4 having yellowish green inside with brownish red and yellowish green in outside, lanceolate (0.5-0.6) by (2.5-3.5) cm, nerves (7-9). Stamens are erect, four in numbers inserted at the base of perianth, brownish red and yellowish green in the apex and basifixed. Ovary is ovoid or ovoid oblong, style absent and stigma is inconspicuous. Fruits are green and ovoid oblong, (1-2.5) by (2-4) cm. Seeds (10-20) are ellipsoid, brown, and 1-1.2 cm long.

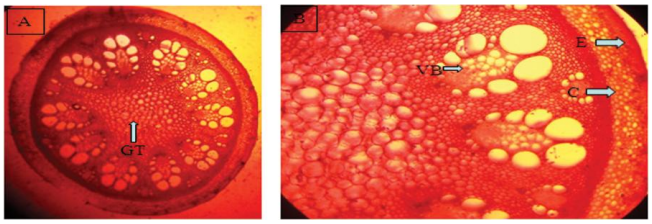

Fig. 2. Photomicrographs of transverse section of *Stemona tuberosa* stem (A, B) showing the vascular bundle (VB), ground tissues (GT), cortex (C) and epidermis (E). Orginal magnification A x 10, B x 40.

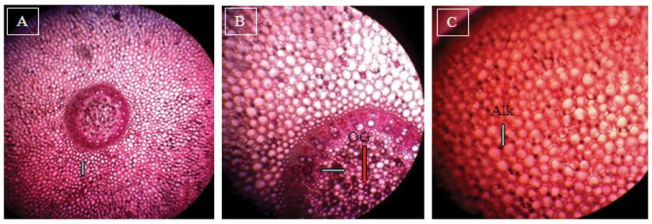

Fig. 3. Photomicrographs of transverse Sections of tuberous root A,B & C showing the deposition of secondary metabolites: oil granules (OG) and crystal structure of Calcium oxalate (CO) and other secondary metabolites like alkaloids (Alk), glycosides etc. Original magnification A x 10, B x 40 & C x 40. Round blackish dots in Fig. C indicates the deposition of alkaloid in the cortex region.

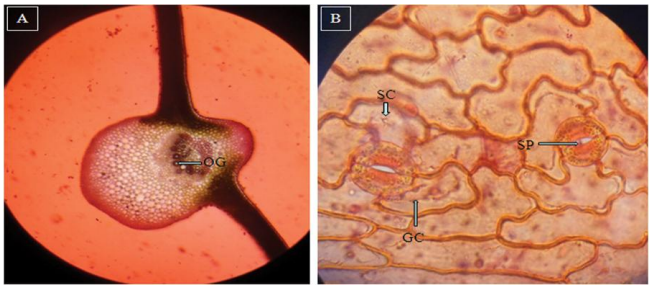

Fig. 4. Photomicrographs of transverse section of leaf (A) & stomata (B). oil granules (OG) are deposited in the leaf mid rib. The stomata is commelina type showing the stomatal pore (SP), Ground cell (GC) and Subsidiary cell (SC). Original magnification A x 10 & B x 40.

Microscopic characters

Transverse sections of both stem (Fig.2 A & B) and tuberous root showed granular and dark crystallized structure which indicating the presence of secondary metabolites and oil drops (Fig.3 A, B, C). Amount of oil granules were found to be more in leaf section compared to root section. Besides that the transverse section of leaf (Fig.4 A) showed high amount of oil drops and crystal like structures determining the presence of various chemical constituents having medicinal and bioactive properties.

Quantitative microscopic character

The stomata is hypostamatous type i.e. only on the lower surface the stomata is present. Stomata (Fig. 4. B) is Commelina type, guard cells surrounded by 4-5 subsidiary cells. The stomatal index is the percentage which the numbers of stomata forms to the total number of epidermal ells, each stomata being counted as one cell. Here on the lower surface the stomatal index is 33.33.

Proximate composition and mineral analysis

Proximate composition of the tuberous root (Table 1) showed the sample to be rich in crude fiber (13.05%) and moisture content (6.082%) whereas crude protein, crude fat and free amino acid showed values of 10.6%, 0.67% and 5.3% respectively. Proteins are Macromolecules that act as alternate energy source when other energy sources are in short supply. They are the building block of any organism. Protein is an essential part of the dietary needs of humans. The reason is that it fulfills a variety of important functions in the body. It is necessary for growth, maintenance and repair of cells and for the production of enzymes and hormones. Furthermore, proteins are the main components of muscle tissue and are vital to the internal organs, bones, skin and the transmission of impulses through the nerves (Sheela, 2004).

Table: 1. Proximate composition of the tuberous root (%)

Parts	Moistur (%)	Ash(%)	Free amino acid (%)	Crude protein (%)	Crude fibr (%)	Crude fat (%)
Powdered root	6.82	2.87	5.3	10.06	13.05	0.67

Fats are vital for a healthy body, provide it with energy, contribute to the absorption of fat-soluble vitamins, and act as structural elements of cell walls. On the other hand, no other nutrient has to combat as many prejudices as fat. It is linked to obesity, type 2 diabetes, cancer, and coronary heart disease (Lichtenstein *et al.,* 1998).

Minerals are essential nutrients, which are said to be present in small amounts in the body or in several parts per million (Gafar and Itodo, 2011). They are essential because they each play important role in metabolic processes of the body and their absence can cause deficiency symptoms in animals (McDonald *et. al.*, 1995; Gafar and Itodo, 2011). Potassium is a key circulating electrolyte which is also involved in the regulation of ATP dependent channels along with sodium. These channels are the Na^+/K^+- ATPases and their primary function is in the transmission of nerve impulses in the brain. Sodium and potassium maintain osmotic and water balance as well as membrane potentials. The Na/K ratio in the body is of great concern for prevention of high blood pressure. Na/K ratio less than one is recommended (Akubugwo *et al.,* 2007). It assists in preventing hypertension and cardiovascular diseases, as dietary potassium is an important cation in regulating blood pressure and attenuating platelet reactivity, which is the major causative factor of vascular occlusion (He and Mac Gregor, 2008). Furthermore, consumption high potassium content enhances the bioavailability of calcium in body and promotes bone health by preventing the occurrences of calciuria. On the other hand, sodium carries an electrical charge and a charged mineral is called an electrolyte. The body regulates the level of sodium in the body through numerous interacting processes because the concentration must remain in a narrow range. If sodium levels deviate too high or too low, it causes problems in the body. Sodium is important for fluid distribution, blood pressure, cellular work and electrical activity. Sodium is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction (Okon and Akpanyung, 2005).

Calcium is an important constituent of bones and teeth and is involved in regulation of nerve and muscle function. In blood coagulation, calcium activates the conversion of pro-thrombin to thrombin. It plays a vital role in enzyme activation. Calcium activates large number of enzymes such as adenosine triphosphatase (ATPase), succinic dehydrogenase and lipase. It is also required for membrane permeability, involved in muscle contraction, normal transmission of nerve impulses and in neuromuscular excitability (Soetan *et al.,* 2010). Reduced extracellular blood calcium increases the irritability of nerve tissue, and very low levels may cause spontaneous discharges of nerve impulses leading to tetany and convulsions (Hays and Swenson, 1985). On the other hand Manganese is a required co-factor for an enzyme called prolidase, which is in turn necessary to make collagen as a structural component of skin. It is also a co-factor for an enzyme called manganese superoxide dismutase (MnSOD), which is a potent antioxidant associated with protection against ultra violet damage. Manganese is needed to help multiple enzymes in a process called gluconeogenesis. This is the process by which we build non-carbohydrate food products, for example, digested fats into sugars to burn as fuel (Gunter *et al.*, 2013). Zinc is found as a co-factor in over 300 different enzymes including antioxidant enzymes. Zinc has a role in the regulation blood glucose levels via insulin function. Zinc is an essential micronutrient for human growth and immune functions (Black, 2003).

Iron functions as haemoglobin in the transport of oxygen. In cellular respiration, it functions as essential component of enzymes involved in biological oxidation such as cytochromes C, C1, and A1 (Malhotra, 1998). Iron is an

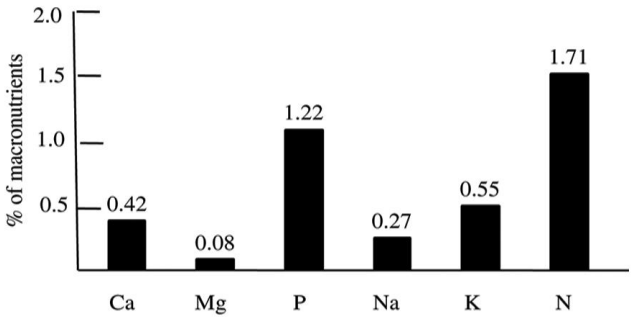

Fig. 5. Macronutrients (%) in the tuberous root.

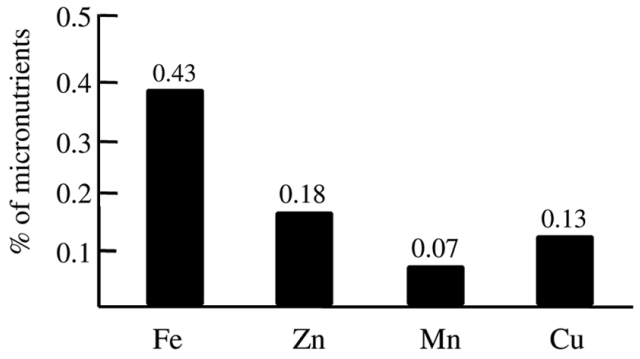

Fig. 6. Micronutrients (%) in the tuberous root.

important constituent of succinate dehydrogenase as well as a part of the haeme of haemoglobin (Hb), myoglobin and the cytochromes (Chandra, 1990). Iron is required for proper myelination of spinal cord and white matter of cerebellar folds in brain and is a cofactor for a number of enzymes involved in neurotransmitter synthesis (Soetan *et al.,* 2010).

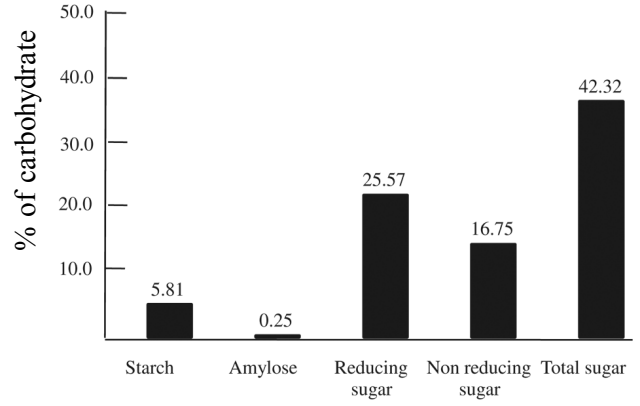

Fig. 7. Carbohydrate content (%) in the tuberous root.

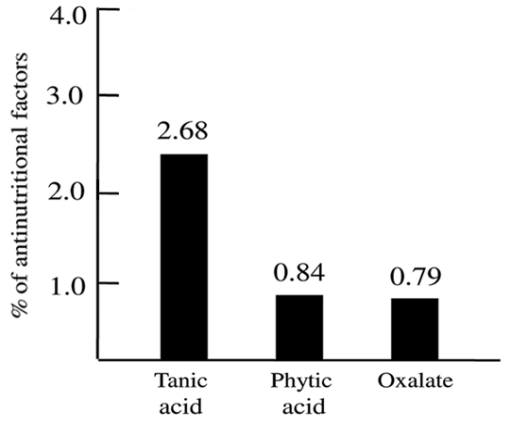

Fig.8. Antinutritional factors (%) in the tuberous root.

Both macro and micronutrients were present in the roots. Among the macronutrients N (1.61%) showed highest and Mg (0.09%) showed the lowest one. Other nutrients like Ca (0.46%), P (1.26%), Na (0.25%) and K (0.54%) etc were also present in a prominent amount (Fig. 5). The result of the analysis of micro mineral content of the tuberous roots of *Stemona tuberosa* revealed that Iron (Fe) content is very high (0.43%), while the value observed for Manganese (Mn), Zinc (Zn) and Copper (Cu) are 0.07%, 0.18% and 0.13% respectively is a moderate one (Fig. 6).

The tuberous root is also rich in carbohydrate contents. Total sugar indicated 42.32% whereas the other starch, amylase, reducing sugar, non-reducing sugar etc were 5.81%, 0.25%, 25.57%, and 16.75% respectively (Fig. 7). The tuberous roots of *Stemona tuberosa* also had the antrinutritional factors like tanic acid (2.68%), phytic acid (0.84%) and oxalate (0.79%) (Fig. 8). Antinutritional facotors reduce the bioavailability of essential nutrients (Binita and Khetarpaul, 1997; Akindahunsi and Salawu, 2005). Aletor and Adeogun (1995) however, reported that some antinutrients exhibit protective effects thus making them serve dual purpose. Oxalate binds to calcium to form calcium oxalate crystals; these prevent the absorption and utilization of calcium by the body thereby causing diseases such as ricket and osteomalacia (Ladeji *et al*., 2004). The calcium crystals may also precipitate around renal tubules causing renal stones. Phytic acid combines with some essential elements such as Fe, Ca, Zn and P to form insoluble salts called the phytates which are not absorbed by the body therefore making these minerals bio-unavailable. Saponins are naturally oily glycosides occurring in wide variety of plants. When eaten, they are non-poisonous to warm blooded animals but are poisonous when injected into the blood stream (Applebaum *et al*., 1969). Tannins are water soluble phenolic compounds with a molecular weight greater than 500 and with the ability to precipitate proteins from aqueous solution. They occur almost in all vascular plants. They combine with digestive enzymes thereby making them unavailable for digestion (Binita and Khetarpaul, 1997).

Constituents	Chemical test	Extracts (Solvents)			
		Chloroform	Acetone	Ethanol	Water
Alkaloids	Hager's test	-	-	+	+
	Myer's test	-	-	+	+
	Wagner's test	-	+	+	+
Carbohydrates and Glycosides	Fehling's test	+	-	+	+
	Benedict's test	-	-	+	+
	Molisch's test	+	-	+	+
Steroids	Salkowski's test	-	-	+	+
Saponins	Foam test	-	-	-	+
Phenols	$FeCl_3$ Sol. test	-	-	-	+
	Lead acetate test	-	-	+	+
Flavanoids	Lead acetate test	+	-	-	-
Proteins & Amino Acids	Biurret test	+	-	+	+
Gums and Mucilage	Alcohol 95% test	-	-	+	+

The tuberous roots of *Stemona tuberosa* were not only rich in only alkaloid content but also rich in other secondary metabolites. Preliminary phytochemical indicated (Table 2) the presence of steroids, polyphenol, glycoside etc. Phenolics are nonnutritive secondary metabolites found in plants that promote significant health benefit and prevent various diseases. Phenols exhibit antioxidant potential (Awika *et al.,* 2003) due to their redox properties which allow them to act as reducing agents, hydrogen donators and single oxygen quenchers (Chang *et al.,* 2001). Flavonol and flavonone are flavonoid of particular importance because they have been found to possess antioxidant and free radical scavenging activity in plants (Amic *et al*., 2003).

Discussion

Traditional medicine is defined by the World Health Organization (WHO, 1978) as the sum total of knowledge or practices whether explicable or inexplicable, used in diagnosing, preventing or eliminating a physical, mental or social disease which may rely exclusively on past experience or observations handed down from generation to generation, verbally or in writing. It also comprises therapeutic practices that have been in existence often for hundreds of years before the development of modern scientific medicine and are still in use today without any documented evidence of adverse effects. Plants, which have formed the basis of sophisticated traditional medicine systems for thousands of years, were originally instrumental to early pharmaceutical drug discovery and industry. Hence, the history of drug discovery and even drug chemistry is inexorably bound to the plant kingdom and the process of deriving drugs from plant sources is certainly not new (Parfitt, 1978).

Ethnobotanical investigation of *Stemona tuberosa* revealed that the tuberous roots are used as antilice, fish poison, preservative and mosquito repellent by certain communities of Arunachal Pradesh. The same uses were also reported from the other parts of the world. (Burkill, 1960; Chuakul, 2000; Agarwal, 2005). The standardization of a crude drug in Pharmacopoeia, pharmacognostic parameters characters serves as an important source of information to ascertain the identity and determination of quality and purity of the plant material for future study (Patil *et al.,* 2012). The presence of oil drops in the leaf and the stem indicated that the plant leaf of *Stemona* is rich in crude vegetable oil. The granular and crystal shaped structure in the root section revealed the presence of secondary metabolites in the roots.

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. That the medicinal actions of plants are unique to particular plant species or groups is consistent with this concept as the combinations of secondary products in a particular plant are often taxonomically distinct (Wink, 1999).

The evaluation of nutrient composition of *Stemona tuberosa* leaf showed that it is highly rich in nutrients and therefore good for human consumption for the maintenance of health and vitality. The roots also showed high carbohydrate content like starch and reducing sugar. Determination of ash value and other proximate compositions can be used as reliable source for detecting adulteration, which helps in the identification of plant materials from the relevant species (Nayak *et al*., 2010). The nutrient and the proximate analysis like carbohydrates, crude proteins, crude fats, crude fibers, moisture, ash (minerals) etc are dominant over anti-nutrient factors which indicated that the plant is a also a good source of food. The anti nutritional factors in general binds to the mineral elements there by forming indigestible complex (Nkafamiya and Manji, 2006) such as oxalate binds with Calcium ion to form complexes (calcium oxalate crystals).

The investigation of phytochemical compounds may be of benefit to the pharmaceutical industries and researchers which is basically based on the knowledge provided by local healers of a particular region (Das *et al.,* 2010). The preliminary phytochemical screening indicated that the roots were not only rich in alkaloid content but also rich in the other secondary metabolites like steroids, proteins, polyphenols etc which revealed their potent therapeutic activity and indicated that the plant may be a source of medicine for curing various diseases (Khandelwal, 2006). Due to the presence of high amount of Stemonine the root extract is used for killing insects and worms. Besides that it also used externally in pediculosis capitis, pediculosis corporis, oxyuriasis (infestation with pinworms) and pudendal itching (Zhu, 1998).

The plant may be considered as a potential source for formulation of useful drugs. Further studies have already been undertaken to isolate, identify, characterize and elucidate the structure of its bioactive compounds. Thus the present study would provide further avenues for carrying out more such studies and sustainable use of plant resources which would also motivate the local communities for adopting effective measures in conservation of useful medicinal plants otherwise subjected to unscientific exploitation and depletion due to habitat destruction. Reasons for the lack of research data involve not only policy problems, but also the research methodology for evaluating traditional medicine. There is a need for validation and standardization of phytomedicines and traditional medical practices so that this sector can be accorded its rightful place in the health care system. As the characteristics and applications of traditional medicine are quite different form western medicine, how to evaluate traditional medicine and what kind of academic research approaches and methods may be used to evaluate the safety and efficacy of traditional medicine are new challenges which have emerged in recent years (Gogtay *et al.,* 2002).

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