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Chlorophyll Content of Some Selected Edible Plants Sourced from Ima Market, Manipur, India

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Abstract

Consumption of fresh green leaves as vegetables or as condiments has been on the rise for their nutritive values and specially to counterbalance the increasing number of degenerative diseases. Chlorophyll which is the most abundant pigments in green plants is of great importance in human diet not only as food colorant but also as healthy food ingredients. Recent research shows that estimating the chlorophyll content could help in understanding the medicinal properties of the plants. Hence, the more the chlorophyll content the more nutritious the leaves will be. In the present study, chlorophyll pigments were extracted and estimated from 19 selected plant species viz: Eryngium foetidum, Allium fistulosum, Allium tuberosum, Allium hookeri, Coriandrum sativum, Plantago major, Zanthoxylum acanthopodium, Houttuynia cordata, Polygonum posumber, Ocimum canum, Brassica juncea, Hibiscus cannabinus, Oenanthe javanica, Ipomoea aquatica, Meyna laxiflora, Mentha spicata, Centella asiatica, Phlogacanthus thyrsiformis and Leucus aspera using 80% acetone, methanol and 95% ethanol as solvents. Results of the study showed that P. posumber has the highest concentration (Chl a 16.977µg/ml, Chl b 7.524 µg/ml) and lowest concentration was noted in Hibiscus cannabinus (Chl a 0.102µg/ml, Chl b 0.336µg/ml) using 80% acetone. It has been found that extraction of chlorophylls by different solvents depends on the plant species as well as on the chemical nature of biomolecules.

Keywords

Chlorophyll, pigment, solvents, biomolecules, species specific.

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INTRODUCTION:

Consumption of fresh green leaves as vegetables or as condiments has been on the rise nowadays specially to counterbalance the increasing number of degenerative diseases. Chlorophyll (a and b) are the most abundant pigments in green plants and are of great importance in human diet not only as food colorant but also as healthy food ingredients (Gaherwar S and Kulkarni P, 2017). Despite being a vital molecule in plants, it acts as blood cleanser, blood builder and also an oxygen booster in the human body. Chlorophyll has great antioxidant capacity and therefore it helps neutralise free radicals and limit oxidative damage within the body.

It is also known to be a source of amino acids which are important to human health. It helps in promoting quick rejuvenation of human cells. It also improves liver functions by extracting the blood impurities. Chlorophyll has been shown to help protect against calcium oxalate build-up which can lead to this form of kidney stone disease. Chlorophyll may help in eliminating or reducing odours associated with urinary disorders and flatulence in the colon. A regular intake of chlorophyll can keep the circulatory and digestive system much healthier. Recent research shows that estimating the chlorophyll content could help in better understanding the medicinal properties of the plants (Kizhedath A *et. al*., 2011). Hence, the more the chlorophyll content the more nutritious the leaves will be.

Manipur, a small state in the north eastern part of India neighbouring to the country of Myanmar, has a long history of using different plants especially as ingredients in the preparation of traditional and indigenous snacks and side dishes such as "Singju", "Eromba" and "Kanghou Bora". The 19 plants listed in this paper are most common, inexpensive and easily available in the local markets. The leaves are most consumed raw and are usually grown in kitchen garden and cultivated commercially.

In the present study, chlorophyll pigments, Chl a and Chl b were extracted and estimated from 19 selected plant species using three different solvents viz. 80% acetone, methanol and 95% ethanol. The concentrations of chlorophyll a and chlorophyll b were compared among the 19 plant samples for their nutritional values. Local people grow these plants in the kitchen garden for daily consumption. Among the 19 plants species selected for study, except for *Brassica juncea* and *Leucus aspera* all the other plants can be consumed as raw. The detailed descriptions and properties of the 19 selected plants are shown in **Table 1**.

Table 1: Detailed Descriptions of the Plants

Name of the plant	Description	Properties
Eryngium foetidum Common name: Mexican Coriander Local name: Awaphadigom	It is mainly grown in most of the places including forests, stream banks, moist places, roadsides etc. Plants are 8–25cm high from a basal rosette.	It is used in traditional medicine for burns, earache, fevers, hypertension, constipation, fits, asthma, stomachache, worms, infertility complications, snake bites, diarrhea, and malaria (Paul *et al*., 2011)
Allium fistulosum Common name: Spring onion Local name: Tilhou mana	It is a monocot perennial plant producing 2-6 hollow, cylindrical leaves 25-40 cm long and a flowering scape 30-50 cm tall from and underground bulb.	The bulb is antibacterial, antiseptic, diaphoretic, diuretic, galactogogue, stomachic, vermifuge and vulnerary. It is one of the Chinese traditional medicines used for the treatment of cardiovascular diseases. (Qinqin FU *et al*, 2010)
Allium tuberosum Common name: Garlic chives Local name: Maroi nakuppi	It is a species of onion native to south western parts of the Chinese province of Shanxi and cultivated and naturalized elsewhere in Asia and around the world. This aromatic herb is capable of flavoring up the dishes most extraordinarily. It is locally available in the state of Manipur and is used as an important condiment or spice in almost all the authentic Manipuri dishes.	Crushed leaves of the herb could be applied to scalp to improve hair growth. The herb is consumed to reduce stress and fatigue. These Chinese chives have long been used as a culinary herb in Asian diets. (Napatsorn *et al*, 2016)
Allium hookeri Common name: Hooker chives Local name: Maroi napakpi	It produces thick, fleshy roots and a cluster of thin bulbs. . The plant is widely cultivated outside its native range and valued as a food item in much of South and Southeast Asia	It contains sulphur compounds which help reduce blood cholesterol levels. The leaves of this plant have been used as a home remedy by Meitei community of Manipur in their folklore medicine as an antiulcer agent. (Khumanthem *et al*, 2018)

Coriandrum sativum **Common name:** Coriander Local name: Phadigom	Major world producers of *C. sativum* include Morocco, India, Pakistan, Romania and the former Soviet Union but coriander is grown in many other countries as well. It is used commonly as aromatic condiment.	It is known to exert antimicrobial activity. Also, a well-known herb widely used as spice in folk medicine and in the pharmacy and food industries. (Filomena *et al*, 2011)
Plantago major **Common name:** Isaphgol Local name: Yempat	It is a species of flowering plant in the plantain family *Plantaginaceae.* It is native to most of Europe and northern and central Asia, but has widely naturalized elsewhere in the world	The leaves of *Plantago major* are effective as a wound healer, as well as an antiulcerative. Leaves and roots are used in fever. Seeds are useful in dysentery, diarrhoea, constipation and genitor-urinary tract complaints. (Sukumar S.S, 2013)
Zanthoxylum acanthopodium **Common name:** Pricky winged leaf Local name: Mukthrubi	It is a species of flowering plant citrus family, *Rutaceae.* It is a prickly, evergreen plant that varies in habit from a shrub to a woody climber or a small tree. It can grow up to 6 m tall	Fruits and seeds used as tonic in fever and dyspepsia and in the treatment of rheumatism, dysentery and stomachache). Fruits used in toothache and scabies. Seeds and leaves used in indigestion, cough and bronchitis. (Virendra S. Rana *et al*, 2007)
Houttuynia cordata **Common name:** Fish mint Local name: Toningkhok	It is a herbaceous perennial plant that can grow from 0.6m spreading upto 1m. The leaves are alternate, broadly heart-shaped, 4-9 cm long and 3-8 cm broad. Its flowers are greenish-yellow and borne on a terminal spike 2-3 cm long with four to six large white basal bracts.	It contains anti-diabetic properties and can be a potential cure for diabetic patients. It can be given to patients suffering from herpes virus since the plant contains properties that are capable of inhibiting pseudorabies herpes virus. It is a well-known traditionally used medicine material in South East Asia (Jiangang Fu *et al*, 2013)
Polygonum posumber **Common name:** Knotgrass Local name: Phakpai	It belongs to the family Polygonaceae. It is grown in garden or wild. People used it in many ways according to their need (both as food as well as medicine)	Crushed leafy shoot is used on forehead against fever. It is used in salads and in local dish (Eromba) of Manipur to increase the aroma. (Sukumar S.S, 2013)
Ocimum canum **Common name:** Wild basil Local name: Mayangton	It is an annual herb with white or lavender flowers. It is native to Africa, Indian Subcontinent, China, South East Asia	It is a diverse and rich source of essential oil. These essential oils are being used as pharmaceutical agents because of their antimicrobial, antidiabetic, antifertility, antistress and anticancer activity. (M. Tamil Selvi *et al*, 2011)
Brassica juncea **Common name:** Mustard leaves Local name: Hanggam	It belongs to the family of Brassicaceae vegetables. It is the largest and most widely consumed a group of plants in Europe and all over the world.	They prevent oxidative stress, induce detoxification enzymes, stimulate immune system and decrease the risk of cancers. They also have antidiabetic, antioxidant, antiatherogenic and astrocyte developing activity. (Ajit Kumar Thakur *et al*, 2014)
Hibiscus cannabinus **Common name:** Kenaf	It is a woody to herbaceous annual, mostly unbranched, fast-growing, with prickly stems, up to 4.2 m tall	Edible oil is yield from this plant and is used as first class cooking oil and margarine production. This oil is nutritionally beneficial to health because of the rich source of bioactive

Local name: Sougri		compounds filled with high antioxidative, anticancer and lipid lowering cholesterol properties. (Sukumar S.S, 2013)
Oenanthe javanica ***Common name:*** Water dropwart Local name: Komprek	It is a perennial herb that belongs to the family Apiaceae. The plant grows wild in freshwater, marshes and swampy fields and along ditches, canals and streams in many Asian countries	Used in the treatment of epidemic influenza, fever and discomfort, jaundice. (Sukumar S.S, 2013)
Ipomoea aquatica Common name: Water Spinach Local name: Kolamani	It thrives in tropical and subtropical regions and is more commonly found in Southeast Asian countries	It helps in the treatment of diabetes, abscesses and intestinal disorders. It also possesses anti-depressant and anti-epileptic activities. (T. Ahemen *et al*, 2015)
Meyna laxiflora ***Common name:*** Muyna Local name: Heibi	It is a small tree or a spinescent. It is found mainly in North-east, West Bengal, Western UP and Deccan Peninsula	Different parts of the plant were used in the treatment of boils, dysentery, diphtheria etc. (Ganesh T *et al*, 2010)
Mentha spicata ***Common name:*** Mint Local name: Nungshihidak	Widely introduced throughout the north temperate zones, *M. spicata* is easily identified by its distinctive odour	It is a commonly used as domestic herbal remedy. Their essential oils are used in cosmetics, perfumes, toothpastes and mouthwashes. (Suleyman *et al*, 2006)
Centella asiatica ***Common name:*** Centella Local name: Peruk	It grows in tropical swampy areas. The stems are slender, creeping stolons, green to reddish-green in colour, connecting plants to each other	It is one of the chief herbs for treating skin problems, healing wounds, revitalizing nerves and brain cells. (Dharmendra *et al*, 2012)
Phlogacanthus thyrsiformis ***Common name:*** Nongmangkha Local name: Nongmangkha	It is found in subtropical Himalayas, from Garhwal to Bhutan and NE India, at altitudes up to 1000 m	Different parts of the plant are being used for the cure of different ailments like fever, gastritis, pharyngitis, cough, bronchial asthma rheumatism and many more. (Sukumar S.S, 2013)
Leucus aspera ***Common name:*** Thumbai Local name: Mayang lembum	It is an erect, annual plant with a much-branched stem 30–60cm tall. It is sometimes cultivated in home gardens for use in local medicine and as a pot herb	The oral consumption of the whole plant is been practiced for antipyretic, analgesic, anti-inflammatory, anti-rheumatic and antibacterial treatment. (Sukumar S.S, 2013)

Materials and methods:

Collection of plants

The selected plant species viz: *E. foetidum, A. fistulosum, A. tuberosum, A. hookeri*, *C. sativum*, *P. major, Z. acanthopodium, H. cordata, P. posumber*, *O. canum, B. juncea*, *H. cannabinus*, *O. javanica*, *I. aquatica*, *M. laxiflora*, *M. spicata*, *C. asiatica*, *P. thyrsiformis* and *L. aspera* were bought from Ima market, Imphal West District, Manipur during the month of June, 2017. Leaves of each plant sample were separated then washed with tap water and finally with distilled water and dried using blotting paper.

Extraction of Chlorophyll

Accurately weighted 0.5g of fresh plant leaf sample were taken and homogenized in mortar and pestle with 10 ml each of the three extractant solvents viz. 80% acetone, methanol and 95% ethanol. Homogenized sample mixtures were centrifuged at 10,000 rpm for 15minutes at 4^{0}C. 0.5ml of the supernatant was taken and mixed with 4.5ml of the respective solvent. The solution mixtures were

analyzed for chlorophyll a and chlorophyll b by observing the absorbance at different wavelengths for the respective solvents using spectrophotometer (ThermoFisher UV 2700) as per the equation given in **Table 2 (**Porra 1989, Lichtenthaler, 1987 and Lichtenthaler and Wellburn, 1983).

Table 2: Equations to determine concentrations (µg/ml) of chlorophyll a (Chl a), chlorophyll b (Chl b) by different extractant solvents in spectrophotometer

Solvent	Equations and formula
80% Acetone	Chl a = 12.7 (A_{663}) – 2.69 (A_{645}) Chl b = 22.9 (A_{645}) – 4.68 (A_{663})
Methanol	Chl a = 16.72 ($A_{665.2}$) – 9.16 ($A_{652.4}$) Chl b = 34.09 ($A_{652.4}$) – 15.28 ($A_{665.2}$)
95% Ethanol	Chl a = 13.36 (A_{664}) – 5.19 (A_{649}) Chl b = 27.43 (A_{649}) – 8.12 (A_{664})

RESULTS AND DISCUSSION

The 19 plant samples selected for our study were categorized into 3 groups based on their usage as below:

1) Condiments (*E. foetidum, A. fistulosum, A. tuberosum, A. hookeri*, *C. sativum*, *P. major, Z. acanthopodium, H. cordata, P. posumber*, *O. canum)*
2) Vegetables (*B. juncea*, *H. cannabinus*, *O. javanica*, *I. aquatica*, *M. laxiflora*) and
3) Medicine (*M. spicata*, *C. asiatica*, *P. thyrsiformis*, *L. aspera).*

Among the plants used as condiments, highest chlorophyll (Chl a and Chl b) content was found in *P. posumber* using 80% acetone as extractant solvent among the three solvents used, followed by *E. foetidum*, *C. sativum*, *A. hookeri*, *A. tuberosum, O. canum, A. fistulosum, H. cordata*, *P. major* and least chlorophyll content was observed in *Z. acanthopodium* using 80% acetone as extractant solvent as shown in Table 3a and Table 3b. In most of the condiments used in our study, chlorophyll content was found to be higher when used with 80% acetone as compared with methanol and 95% ethanol. Whereas in *Z. acanthopodium* chlorophyll content was found to be highest using methanol as extractant solvent as compared with 80% acetone and 95% ethanol. But in *P.major* Chl a content was found to be high est using methanol as extractant solvent and Chl b using 95% ethanol.

Among the five vegetables, highest chlorophyll content was observed in *B. juncea* followed by *O. javanica*, *I. aquatica*, *M. laxiflora* and least chlorophyll content in *H. cannabinus* using 95% ethanol (Table 4). Results also indicate that 95% ethanol was the most favourable solvent for extraction of chlorophyll from five plant species except *O. javanica* for which 80% acetone was found to be the best solvent for chlorophyll extraction.

Among the four medicinal plants, highest extraction of chlorophyll was noted in *L. aspera* and least in *P. thyrsiformis* using 95% ethanol as extractant solvent (**Table 5**). Whereas for *M*. *spicata* and *C. asiatica*, 80% acetone and methanol were found to be the best extracting medium respectively.

P. posumber has also been noted to have the highest chlorophyll content using 80% acetone among all the selected plants used for our study (**Fig.3**) while least was observed in *H. cannabinus*. Using methanol as extractant solvent, *P. major* has been noted to have the highest chlorophyll content among all the selected plants as shown in **Fig 4** while least was observed in *P. thyrsiformis*. However, *I. aquatica* was found to have highest chlorophyll content among all the plants when 95% ethanol was used as extractant solvent and least in *O. canum* as shown in **Fig 5**.

The variations in chlorophyll content among the selected plant samples of our study for same extractant solvent may be attributed to inherent physiological characteristics of the individual species. Temporal and seasonal changes and local geological condition can also be the reason for variations in pigment concentrations in plants (Sumanta *et al*, 2014).

Table 3a: Chlorophyll content of plants mostly used as condiments

Extractant Solvent	Eryngium foetidum (µg/ml)		Allium fistulosum (µg/ml)		Allium tuberosum (µg/ml)		Allium hookeri (µg/ml)		Coriandrum sativum (µg/ml)	
	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b
80%Acetone	13.427	5.655	3.18	1.173	7.412	2.198	7.156	3.214	11.512	4.193
Methanol	7.148	3.111	2.116	0.715	2.85	1.176	2.71	0.943	6.962	2.305
95% Ethanol	5.435	2.300	2.785	0.905	3.736	1.686	3.474	1.384	8.521	2.832

Table 3b: Chlorophyll content of plants mostly used as condiments

Extractant Solvent	Plantago major Linn (µg/ml)		Zanthoxylum acanthopodium (µg/ml)		Houttuynia cordata Thunb (µg/ml)		Polygonum posumber Berch (µg/ml)		Ocimum canum Sims (µg/ml)	
	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b
80%Acetone	1.197	1.289	0.846	1.484	2.078	1.404	16.977	7.524	6.796	3.169
Methanol	16.733	1.159	11.520	2.896	6.028	2.541	12.925	6.165	3.505	2.330
95% Ethanol	2.141	1.489	5.867	2.793	0.953	0.585	2.905	2.142	0.573	0.719

Table 4: Chlorophyll content of plants mostly used as vegetables

Extractant Solvent	Brassica juncea (µg/ml)		Hibiscus canabinus (µg/ml)		Oenanthe javanica (µg/ml)		Ipomoea aquatica (µg/ml)		Meyna laxiflora (µg/ml)	
	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b
80%Acetone	5.365	2.010	0.102	0.336	9.614	3.073	0.423	0.325	6.054	2.041
Methanol	7.297	3.026	1.343	2.090	8.783	2.800	4.452	2.205	5.402	2.521
95% Ethanol	9.052	4.087	4.680	1.694	8.110	2.814	9.091	3.068	7.368	2.609

Table 5: Chlorophyll content of plants mostly used as medicines

Extractant Solvent	Mentha spicata (µg/ml)		Centella asiatica (µg/ml)		Phlogacanthus thyrsiformis (µg/ml)		Leucas aspera (µg/ml)	
	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b
80% Acetone	4.909	2.265	2.315	1.184	0.866	0.688	6.232	2.282
Methanol	4.240	1.962	5.976	2.356	0.610	0.779	7.483	2.805
95% Ethanol	4.155	0.596	4.093	1.735	0.733	1.059	8.404	3.072

Fig 1: Nineteen selected plants for the study

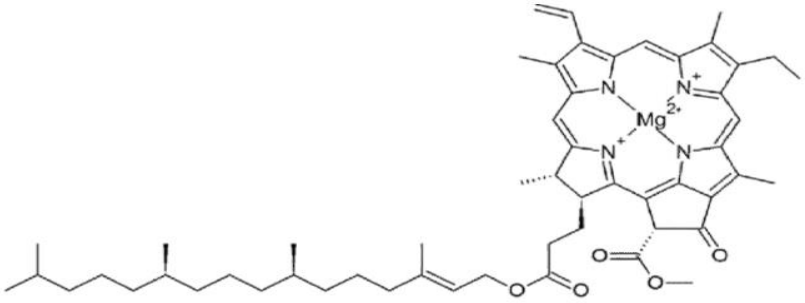

Fig 2: Chemical Structure of Chlorophyll

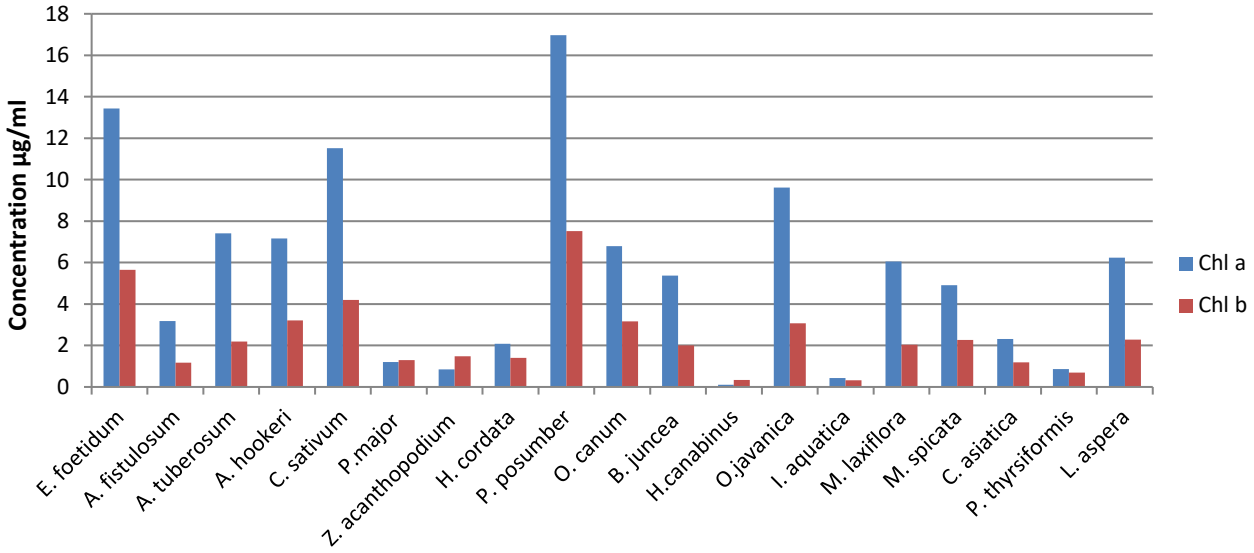

Fig 3: Chlorophyll concentrations of the selected plants using 80% acetone

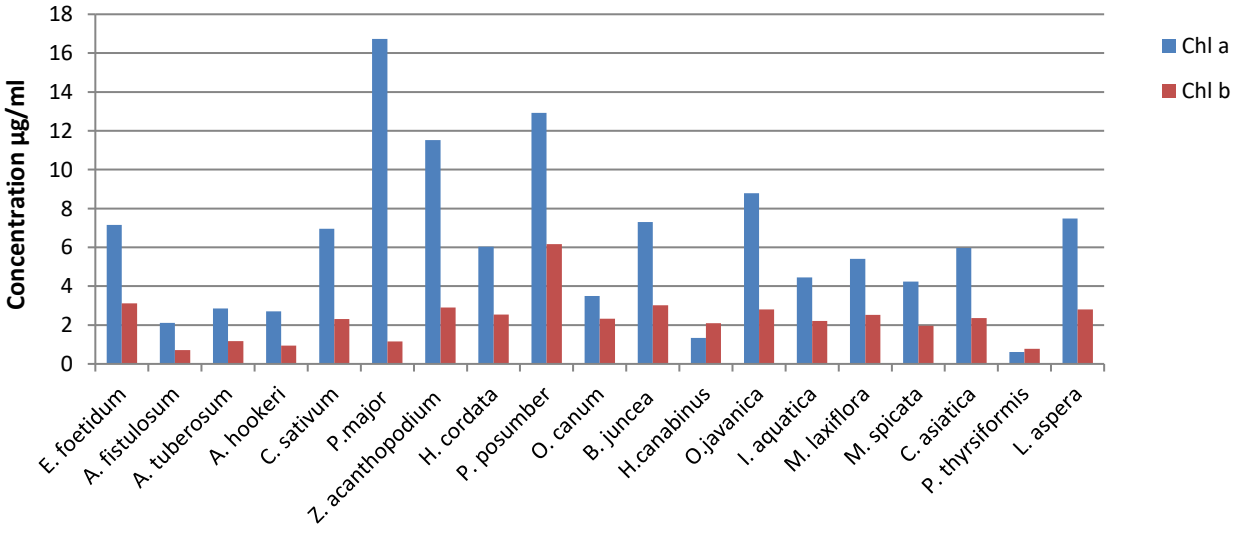

Fig 4: Chlorophyll concentrations of selected plants using methanol

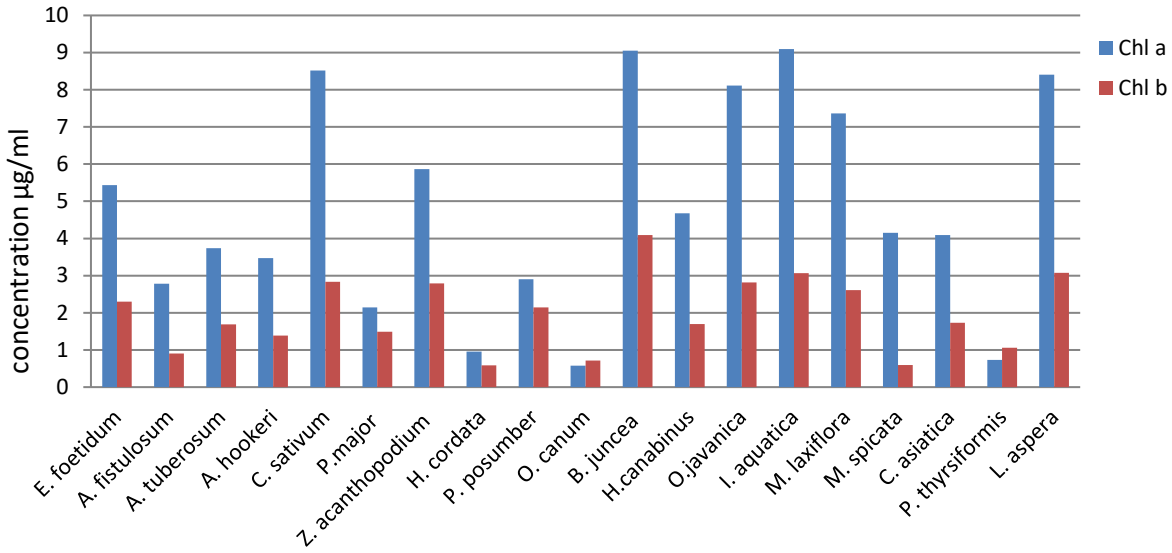

Fig 5: Chlorophyll concentrations of selected plants using 95% ethanol

CONCLUSION

Plants are an integral part of our lives. The chlorophyll provides alkaline environment in human body in addition to be an oxygenator therefore helping our bodies fight against disease causing bacteria. Chlorophyll helps the body to cleanse itself of toxic substances. In larger perspective it is linked with cancer prevention through its cleansing roles. In Manipur, from time immemorial, people used herbs while preparing the traditional dishes especially vegetarian to increase the flavor and aroma. *E. foetidum, A. fistulosum, A. tuberosum, A. hookeri* and *C. sativum* are produced throughout the year in Manipur. *P. major, Z. acanthopodium, H. cordata, P. posumber*, *O. canum* are mostly consumed raw and they are known for their flavor and aroma. *B. juncea, H. cannabinus, O. javanica, I. aquatica* and *M. laxiflora* are among the most consumed vegetables by the people of Manipur. *M. spicata, C. asiatica, P. thyrsiformis, L. aspera* are also used as vegetables and are known for their medicinal values. Locals fof Manipur consume these plants either in fresh or in cooked form.

From the result, it can be concluded that among the 19 plant samples studied, *P. posumber* has the highest chlorophyll content and *H. cannabinus* has the lowest using 80% acetone as extractant solvent. It was also found that with difference in plant species, the suitability of solvents used in extracting chlorophyll also differed. It has been found that extraction of chlorophyll by different solvents depends on the plant species as well as on the chemical nature of biomolecules.

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