POLLEN MORPHOLOGY, PHYTOCHEMICAL TEST AND ANTIMICROBIAL ACTIVITIES OF TEA LEAVES FOUND IN WAN SAING VILLAGE, KYAING TONG

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Abstract

From the methanolic extract of green tea leaves of *Camellia sinensis* (L) Kuntze, collected from *Wan - Saing*, a *Loi* (ethics minority in Myanmar) village within the Tract of *Kat- Pha*, Kyaing Tong District, Eastern of Shan State. Phytochemical contents of the tea leaves were analyzed and evaluated. By these analyses, carbohydrate, glycoside, phenolic compound, α - amino acid, saponin, tannin, flavonoid, steroid, terpenoid and reducing sugar are found to be present. Notably, starch and cyanogenic glycosides were not observed in the extract sample under study. In addition the values of the nutrients from the leaf extract were examined using AOAC (Analytical Official of Chemic Method). As a result the energy value was found to be the highest with the potential value of 280 % whereas carbohydrate content is second in line with the concentration of 51.33%. The concentric percent of the remaining nutrients such as moisture, ash, crude protein, crude fiber and crude fat were found to be 11.73%, 5.02%, 10.42%, 17.80% and 3.70% respectively. Caffeine concentration was found as the lowest in concentration (0.27%). By agar-well diffusion method, using different kinds of solvents, the antimicrobial activity of constituents from the leaf extract were studied. Their inhibitory zones were also evaluated.

Keyword: pollen morphology, phytochemical tests, nutritional values and antimicrobial activities, Wan Saing Village, Kyaing Tong Township.

Introduction

The green tea made from leaves of Camellia *sinensis* (L.) Kuntze is the second most (after coffee) consumed beverage in the world. The tea bush grows best on well- drained acid habitat. Soil in a warm climate with ample rainfall of about 150 – 300 cm (60-200 in) per year. The tea plant in nature is a small tree but grown under cultivation it is intentionally kept under 2 m (3-5 ft) to enable pickers to harvest the young leaves. . Constant pruning stimulate the vigorous development of new shoot called "flushes". Harvesting is usually done by hand to ensure the best quality.

The quality is dependent on a number of factors: cave taken in cultivation, rain fall and elevation at which it was grown. The flavour and quality vary with the soil type, climate, age of the leaf, time of picking and method of preparation. In fact, the green tea itself is actually the dried which leaves are dull green with even texture.

There are three main types of tea: (i) green tea where the leaves are steam-dried without being allowed to ferment; (ii) black tea, where the leaves are fermented and dried; (iii) among Myanmar people there is still another type of tea consuming-pickled tea, where the tea leaves are steam- dried and fermented. As the saying goes " The king of the leaf is pickled tea leaf" consuming together with a variety of fried dried beans make the green tea the most palatable of all of food delicacies.

The significance of pollen attributes in taxonomy has been widely realized. Recently palynological data are found to be applicable critical in advance investigation on angiosperm

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flora. Diagnoses base on pollen features have been found in agreement with those prepare on the basic of Botany (Metcalfe, 1961).

The classification of pollen is base on number position character analysis known as "NPC" system. Due to the fact that pollen morphology should have been property appraised in angiosperm systematic recent systems of classification proposed by Zimmer Mann and Cronquist. Palynological characteristic were provided and used as one of the main criteria in identification of the plant sample.

Thirteen bioactive components of green tea leaves have been detected the phytochemical test showed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, α-amino acids, saponins, tannins, flavonoids, steroids, terpinoids and reducing sugar as the leaf components where as the starch and cyanogenic glycosides were totally absent.

When the nutritional value is considered, the following characteristics tendencies were observed: the energy content was found as the most abundant; caffeine was the leaf content of all with the value 0.27 in percent. The value of the remaining components were observed in the following sequence; Carbohydrate (51.33 %); Moisture (11.73%); Ash (5.02 %); Protein (10.42 %); Fiber (17.80 %); Fat (3.70 %).

Six different kinds of solvents were prepared for the detection of antimicrobial activity. This experimentation was conducted using agar – well diffusion method. The results showed that-

(i) The ethanol extract had the most efficient impact against all the six species of the microbes under study especially against *Bacillus pumilus* and *Escherichia - coli* with the maximum record inhibition zone of 21 mm.

(ii) The ethanol – water extract also showed the impact upon all the six microbes but with the inhibitory zone a bit limited in area.

(iii) The methanol extract showed to possess the inhibitory effect upon the other five microbe species except *Pseudomonous aeruginosa*.

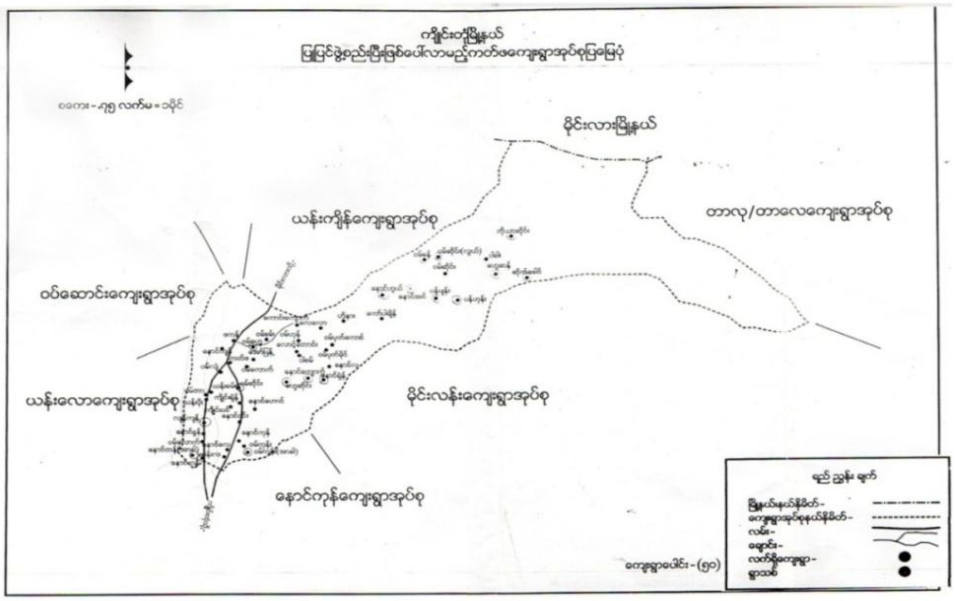
The present study was carried out to investigate the leaves of *Camellia sinensis* (L.) Kuntze, for medicinal and health for human being. It can be assume that the study result will be helpful to students or investigator who interested in morphology, nutritional value, phytochemical and antimicrobial activities.

Materials and Methods

1. Collection and plant systematic study

The green tea leaves of *Camellia sinensis* (L.) Kuntze were cultivated in *Wan-Sai*, village of *Loi* ethnics people, *Ka-Pha* tract, Kyaing Tong District. The specimens were collected during September to December, 2018 at the period of anthesis and fructification. Natural habit and flowers had been photographed. The plant material was identified and specimens are deposited in our Department Herbarium, University of Kyaing Tong.

Location map of study area



2. Collection of pollen samples

A few stamens of *Camellia sinensis* (L.) Kuntze were taken from buds (than in full bloom flowers). Collected pollens was stored in small glass vials with 1 cc of glacial acetic acid and labeled.

2 (a) Acetolysis of reference material

Polliniferous material was crushed with a glass rod, 1cc of glacial acetic acid was added and then 3 to 5 drops of concentrated sulphuric acid was added to the above. The test tube was put in a water-bath for 15 to 30 minutes. The fluid in the test tube was stirred frequently and boiled. On cooling, the mixture was diluted with distilled water to centrifuge for 15-30 minutes. This was repeated twice, decanting the water each time. After centrifuging, the distilled water was removed and glycerine jelly with saffranin was added to the polliniferous material and this was then transferred to store in air-tight bottle and labeled.

2 (b) Preparation of glycerine jelly

It is prepared by Kisser's formula (Erdtman, 1952), using 50 gms of gelatine, 150 ml of glycerine and 7 gms of phenol crystals were mixed with 175 ml of distilled water in a beaker and stirred with a glass rod. This was heated for about 3 hours in a water bath till homogenous. Then 0.05g of saffranin was finally added just before removal and stored. Only the amount of material needed during the day should be made each time.

2 (c) Preparation of microscopic slides

The sample bottle was warmed in a water bath and a drop of polllliniferous jelly was taken out with a pair of forceps and placed on the glass slide, and then covered with a cover slip. The pollen sample was examined by using electric Novex Trinocular Microscope with 40 X and imaged by taking Canon (8.0 megapixels) Digital Camera. A micrometer was used to measure the size of the grain.

3. Phytochemical Examination of leaves of *Camellia sinensis* (L.) Kuntze

The dried leaves of green tea plant *Camellia sinensis* (L.) Kuntze were cut and repeatedly washed with water. The slice samples were dried under shades for three weeks. Dried samples were ground into powder form for phytochemical investigation.

Phytochemical test of the sample has been conducted to at Food Industries Development Supporting Laboratory (FIDSL), Myanmar Food Processors and Exporters Association (MFPEA), Yangon.

The leaf powders were extracted 3 times with methanolic from the methanolic extracts, 13 major photochemical viz, alkaloids, carbohydrates, glycosides, phenolic compounds, α-amino acids, saponins, tannins, flavonoids, steroids, terpinoids, reducing sugar, starch and cyanogenic glycosides were isolated and analyzed.

4. Nutritional value investigation

The nutritional value of the leaves of *Camellia sinensis* (L.) Kuntze were identified according to analytical official of chemic methods (AOAC), at Food Industries Development Supporting Laboratory (FIDSL), Yangon.

5. Antimicrobial activities

The samples were dried and powdered. The leaf powder was subjected to examine antimicrobial activities by Ager-well diffusion method after Cruickshank, 1970.The crude sample was subjected to antibacterial screening against some pathogenic organisms. These organisms were *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albicans* and *Escherichia coli*. These investigations were conducted at the Pharmaceutical Research Department of Ministry of Industry Yangon Division.

Results

I. (a) Systematic Position of the Tea plant

Kingdom	- Plantae
Division	- Magnoliophyta
Class	- Magnoliopsida
Order	- Theales
Family	- Theaceae
Genus	- *Camellia* L.
Species	- *Camellia sinensis* (L.) Kuntze
Myanmar name	-Laphet
English name	-GreenTea
Loi name	-Larce^
Shan name	-La

I. (b) Taxononic Description

Shrubs or trees, stem and branches cylindrical, internodes 2.0 to 3.5 cm long, 2.0-4.0 mm wide, grayish-brown, white-pubescent. Leaves simple, alternate, exstipulate, petiolate; petiole terete, 4.0-7.0 mm, green, pubescent, glabrescent. Blade oblong- elliptic, leathery 5.0-16.0 cm long and 2.0-8.0 cm wide, abaxially pale green and glabrous, adaxially dark green, shiny and

glabrous midvein, serrate to serrulate along the margin acute to acuminate at the apex, with an obtuse tip, cuneate to broadly cuneate at the base. Lateral nerves 5 – 7 on each side of midvein, reticulate veins visible on both surfaces. Flowers bisexual, actinomorphic, hypogynous, white, axillary, solitary 2 – 4 cm across at anthesis, ebracteate, bracteolate, pedicellate; pedicel 5.0 -10.0 mm, recurved pubescent or glabrous, thickened toward apex. Bracteoles 2, caduceus, ovate; sepal 5-6, broadly ovate to suborbicular. Petals 5-6, white outer 1-3 petals sepaloid; inner petals obovate to broadly obovate. Ovary globose, sparsely white pubescent, 3-loculed; style linear, glabrous or base pubescent, apically 3-lobed. Capsule oblate, rarely globose, 1-3 loculed with 1-2 seeds per locule; pericarp about 1mm thick. Seeds subglobose, brown .

Flowering and fruiting period : September to December

A distant view of Tea Plantation on mountain slop

Habit

A tall tea plant understudy

Tea leaves

Flower

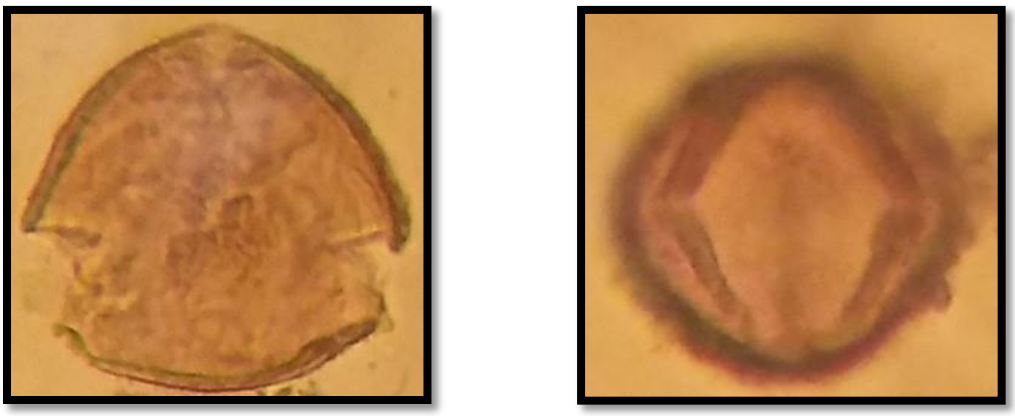
Fruits

Figure 1 Morphological characters of *Camellia sinensis* (L.) Kuntze.

2. Pollen morphology of *Camellia sinensis* (L.) Kuntze.

The present research concerns the palynology of *Camellia sinensis* (L.) Kuntze, of Family Theaceae. The palynological data is illustrated with figure as follow-

Aperture condition tricolporate, medium size, pollen shape oblate, 25-30 X 25-35µ in length and breadth; amb subtriangular; polarity isopolar; colpi longicolpate, about 20-30 x 3-5 µ in length and breadth; pori lolongate about 3.5 x 2.5 µ in length and breadth; exine thicker than nexine; sculpturing verrucate.



Polar view Equatorial view

Figure 2 Pollen Morphological characters of *Camellia sinensis* (L.) Kuntze.

3. Phytochemical examination of leaves of *Camellia sinensis* (L.) Kuntze.

Preliminary phytochemical tests of the leaves of Tea plant indicated the experimental result was shown in table (1).

Table 1 The Phytochemical examination of *Camellia sinensis* (L.) Kuntze.

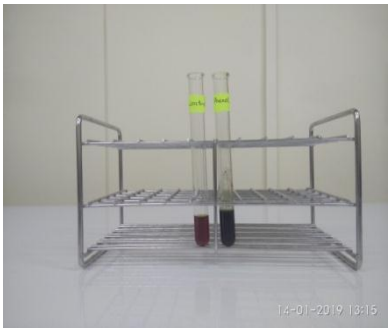
No.	Type of compound	Extract	Reagent used	Observation	Results
1.	Alkaloid	1%HCL	Mayer's reagent	Cream colour ppt.	+
			Wagner's reagent	Brown ppt.	
			Dragendorff 's reagent	Reddish brown ppt.	
			Hager's reagent	Yellow ppt.	
2.	Carbohydrate	H_2O	10% α -naphthol & H_2SO_4 (Conc:)	red ring	+
3.	Glycoside	H_2O	10% Lead acetate solution	White ppt.	+
4.	Phenol	H_2O	5% $FeCL_3$ solution	Greenish black ppt.	+
5.	α-amino acid	H_2O	Ninhydrin reagent	Pink colour	+
6.	Saponin	H_2O	H_2O	Persistent foam	+
7.	Tannin	H_2O	1% Gelatin & 10% NaCL solution	ppt.	+
8.	Flavonoid	70%EtOH	Mg ribbon & Conc; HCL	Pink colour	+
9.	Steriod	Petroleum ether	Acetic anhydrite & Conc; H_2SO_4	Bluish green	+
10.	Terpenoid	Petroleum ether	Acetic anhydrite & Conc; H_2SO_4	Pink	+
11.	Reducing sugar	H_2O	Fehling's solution	Brick red ppt.	+
12.	Starch	H_2O	Iodine solution	Brown ppt.	-
13.	Cyanogenic glycoside	powder	H_2O,Conc; H_2SO_4 sodium picrate paper	No colour change	-

(+) = present (-) = absent

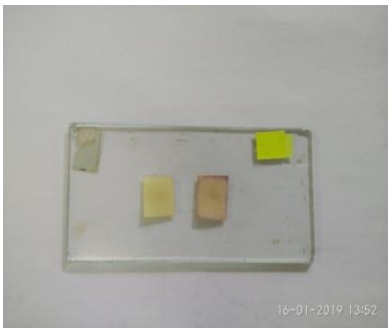
Test for Alkaloid

Test for Carbohydrate

Test for Glycoside



Test for Phenol



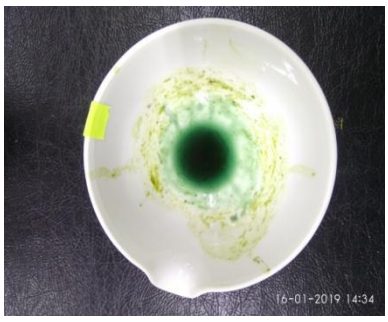
Test for α-Amino acid

Test for Saponin

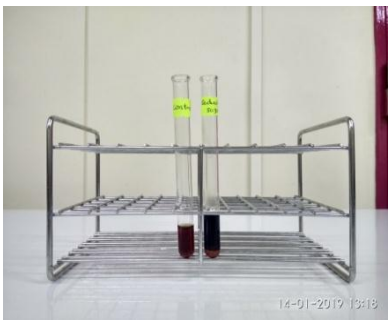
Test for Tanin

Test for Flavonoid

Test for Steroid

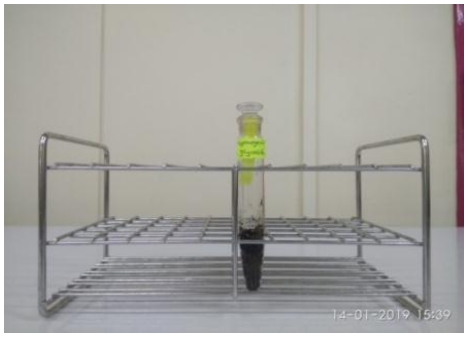


Test for Terpenoid



Test for Reducing sugar

Test for Starch




Test for Cyanogenic glycoside Thirteen kind of Phytochemical test

Figure 3 Test for Phytochemical examination of *Camellia sinensis* (L.) Kuntze.

4. Nutritional value of leaves of *Camellia sinensis* (L.) Kuntze,

Determination of nutritional value carried out at the laboratory of Food Industries Development Supporting Laboratory (FIDSL). The result of the detection showed that the energy content was found to be the most abundant, the value of carbohydrate and the remaining nutrients are as shown in table (2).

Table 2 Nutritional value of leaves of *Camellia sinensis* (L.) Kuntze

No	Type of Nutrients	Content (%) of leaves of *Camellia sinensis* (L.) Kuntze
1	Moisture	11.73 %
2	Ash	5.02 %
3	Protein	10.42 %
4	Fiber	17.80 %
5	Fat	3.70 %
6	Carbohydrate	51.33 %
7	Energy (Kcal / 100g)	280 %
8	Caffeine	0.27 %

5. Antimicrobial activities of leaves of *Camellia sinensis* (L.) Kuntze

Antimicrobial activities were conducted at the Pharmaceutical Research Department of Ministry of Industry, Yangon Division.

Table 3 Test for Antimicrobial activities of leaves of *Camellia sinensis* (L.) Kuntze

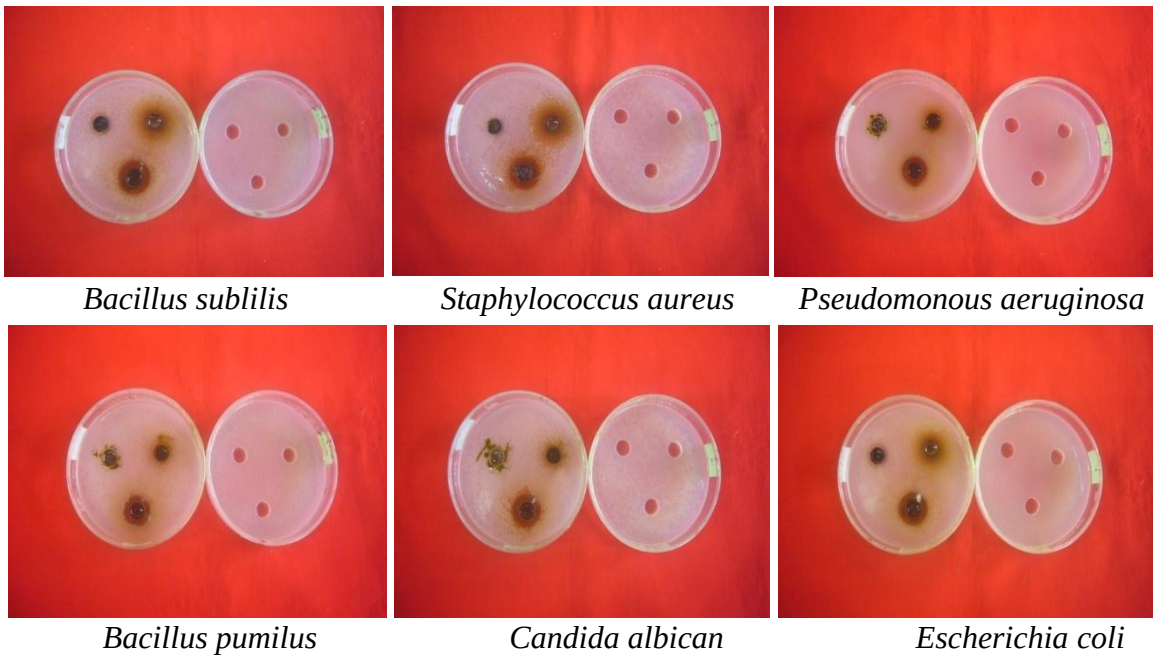
Samples	Solvents	Organisms					
		Bacillus subtilis	***Staphylo coccus aureus***	***Pseudomo-nas aeruginosa***	***Bacillus pumilus***	***Candida albican***	***E-coli***
Tea Plant	Pet-ether	-	-	-	-	-	-
	Methanol	19 mm (++)	20 mm (+++)	-	20 mm (+++)	20 mm (+++)	18 mm (++)
	Acetone	12 mm (+)	-	-	14 mm (+)	15 mm (++)	14 mm (+)
	Ethanoic acid	15 mm (++)	-	-	15 mm (++)	15 mm (++)	16 mm (++)
	Ethanol	19mm (++)	20 mm (+++)	20 mm (+++)	21 mm (+++)	17 mm (++)	21mm (+++)
	Water	17 mm (++)	15 mm (++)	17 mm (++)	16 mm (++)	17 mm (++)	16 mm (++)

Agar well – < 10 mm (inactive), 10 mm ~ 14 mm (partially active), 15 mm ~ 21 mm (active)

Organisms

Agar well – 10 mm
10 mm ~ 14 mm (+)
15mm ~ 19 mm (++)
20 mm above (+++)

(1) *Bacillus sublilis*
(2) *Staphylococcus aureus*
(3) *Pseudomonous aeruginosa*
(4) *Bacillus pumilus*
(5) *Candida albican*
(6) *Escherichia coli*



Bacillus sublilis *Staphylococcus aureus* *Pseudomonous aeruginosa*

Bacillus pumilus *Candida albican* *Escherichia coli*

Figure 4 Antimicrobial treatment of different solvent extracts of leaves of *Camellia sinensis* (L.) Kuntze

Discussion and Conclusion

Man has always sought to make his drinks more palatable to taste than is pure water. The diffusion and decoctions from variously processed plant parts; especially the tea and coffee are the most commonly used today. Tea is obtained from the tea plant, *Camellia sinensis* (L.) Kuntze. The naturally growing tea plants can be tall trees upon the height of 15-20ft. But they are usually heavily pruned and not allowed to reach more than 1.5 - 2.0 meters in height. Harvesting is traditionally done by hand to ensure the best quality. The tea plantation from which we collected the plant samples is located at the attitude of about 5000-5700 feet and above.

It is a large plantation cultivated upon steep ridges and mountain slopes with soil that is too poor for other types of agriculture can thrive on. The tea plant is propagated from seeds or from seedling. In the above said plantation apart from low shrubs for commercial purposes. There are some two hundred tall plants of about twenty feet. These plants continue growing for 50 years or more. It is said that the tea plants in their grove are propagated from seeds of a century old plants at their farm. The flavor of the processed tea is dependent on the content of the plant's phytochemical components. The stimulating effects are the result of its caffeine content.

The Phytochemical test of the leaf powder of the plant sample indicated that carbohydrate, glycoside, phenolic compound, α-amino acid, saponin, tannin, flavonoid, steroid, terpenoid and reducing sugar are present but that starch and cyanogenic glycoside were not observed.

From the view point of the nutritional value, the Energy (Kcal / 100) value was found to be the highest (280%) and the Carbohydrate (51.33 %), runs second whereas Moisture (11.73%), Ash (5.02 %), Protein (10.42 %), Fiber (17.80 %), Fat (3.70 %) and Caffeine was found to be the content the least (0.27 %) in this experiment.

To examine antimicrobial activities, leaf extract in six different kinds of solvent namely petroleum ether, methanol, acetone, ethanoic acid, ethanol and water were used by agar well diffusion method. The experiment revealed that the ethanol extract showed the maximum inhibitory effect with 21 mm inhibition zone, in ethanol extract against the *B pumilus* and *E. coli*. The ethanol-water extract showed antibacterial activity against all the six species of organism. The methanol extract showed antibacterial activity against the other five species except *Pseudomonous aeruginosa.* Acetone and Ethanoic acid extract showed antibacterial activity against other four species except *Staphylococcus aureus* and *Pseudomonous aeruginosa.* Pet ether extract do not showed the antibacterial activity.

The usefulness and applicability of pollen investigations in plant systematic should have been considered because many pollen traits are influenced by the strong selective forces evolved in various reproductive processes including pollination, dispersal and germination. In plant systematic criteria of pollen are of the pollen size, pollen shape, pollen type, structure of the pollen wall, pollen architecture, and number of aperture, aperture position and aperture shape. In this study, tricolporate, medium size, pollen shape oblate, amb subtriangular, polarity isopolar, exine thicker than nexine and sculpturing verrucate were found as the pollen charactreristics.

In this respect the authors like to suggest that the further advance investigations focusing on phytochemistry and physiology of the plant species are needed.

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