AUTHENTICATION OF SOME COMMERCIAL TEAS IN ILORIN, NORTH-CENTRAL, NIGERIA

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Adulteration and substitution of herbal drugs are trending issue in the herbal industry, posing a serious threat to commercial natural product research. Anatomical and chemical studies were carried out on *Camellia sinensis* and 6 commercial tea samples using chemical maceration technique (C-tea, L-tea, T-tea, H-tea, N-tea and A-tea). *C. sinensis* has hypostomatic leaves with paracytic and anomocytic stomatal complex types and thick-cell wall. The plant also possessed non-glandular unicellular trichomes. The anticlinal wall pattern was straight and rectangular, and it is undulating on the adaxial surface. Anatomically, C-tea has paracytic and pericytic stomatal complex types and unicellular non-glandular and multicellular glandular trichomes. L-tea has paracytic and anomocytic with unicellular glandular and multicellular non glandular trichomes. Paracytic, polocytic and anisocytic stomatal complex types with unicellular glandular and multicellular non-glandular trichomes were observed in T-tea. Occurrence of paracytic and anomocytic stomata was observed in H-tea; paracytic and tetracytic stomata were present in N-tea while paracytic stomata were observed in A-tea with unicellular glandular trichomes. The chemical tests shown that Prussian blue was present in three commercial samples of black tea and turmeric was present in two commercial green tea samples. Anatomical studies revealed that four of the selected commercial tea samples shown traces of adulteration.

KEYWORDS: Commercial Tea, *Camellia sinensis*, Stomatal type, Trichomes and Adulterations.

INTRODUCTION

Adulteration is commonly defined as the intentional or unintentional addition or subtraction of any substances to or from food or medicinal products, so as to affect their natural composition and quality (Nidhi *et al*., 2009). Tea (*Camellia sinensis* (L.) O. Kuntze) is an ancient crop that has been cultivated for thousands of years (Jianwei *et al*., 2016). Tea adulteration is a common scenario, affecting not only the nature, substances and quality of tea, but also their incidental contamination during the period of growth, harvesting, storage, processing, transport and distribution. Adulteration means any material

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which is or could be employed for making the tea product unsafe, substandard, miss-branded or containing extraneous matter (Rappaport, 2006).

The commonest problem faced by herbal drug preparation in herbal is the correct identification of genuine raw materials; in the absence of relevant data, one can use adulterants in the drug formulation (Soni *et al*., 2008). It is observed that the plant collectors and traders add or replace the original materials with the cheaper plant materials especially as in case of tea (Sultana, 2012). It is not a case of inadvertent mistake but a malpractice, resulted into the adulteration, which in turn decreases the quality and therapeutic value of the herbal drug. In case of *C. sinensis,* Sultana (2012) noted that the leaves and aerial parts of *Chenopodium ambrosoides* are substituted. Other examples of such adulterations can be cited with regard to the bark of *Cesalpinia sappan*, where the bark of *Pterocarpus* and *Toonaciliata* are substituted (Purohit *et al.*, 2004).

Tea leaves which get damaged during manufacturing and those with inferior quality are being treated with various colouring agents to improve their appearance and price (Teshome, 2019). Adulteration of tea leaves is done by treating processed leaves with a mixture containing Prussian blue, turmeric or indigo etc (Rappaport, 2006). Black teas are usually treated with plumbago (black lead) which is used in making lead pencils (FSSAI, 2011). Adding foreign material(s) to teas for the purpose of deception should be strongly discouraged. Scientific studies are required to evaluate the impact of using colour to human health (Spence, 1976).

The study was conducted to determine the leaf anatomical features and chemical contaminants of *C. sinensis* and some commercial tea samples which could serve as a tool for identification of adulteration in the 6 selected commercial tea samples.

MATERIALS AND METHODS

Collection of study materials

The fresh matured leaves of *C. sinensis* were collected from Cocoa Research Institute of Nigeria (CRIN), Ibadan, Oyo State, Nigeria. Six commercial samples of green and black teas were purchased from two major markets in Ilorin, Kwara State, Nigeria (Table 1).

Isolation and microscopic study of epidermal layers of Camellia sinensis

Leaf segment of an area of 1sqcm were macerated in 10% concentrated nitric acid at the room temperature for 48 hours. The upper and lower epidermal surfaces were separated by using dissecting needle and forceps.

A small portion of macerated epidermal layers was stained in 1% aqueous solution of safranin for 3 to 5 minutes. Excess stain was rinsed off with distilled water. The specimen was then mounted in glycerine for microscopic study to determine stomatal complex types, epidermal cell shapes and trichomes. Photomicrographs were taken with Amscope digital camera attached to the light microscope.

Microscopy study of commercial tea samples

One (1) gm. of commercial tea samples was boiled with 50 ml of 2% sodium hydroxide for about 2–3 minutes. The mixture was diluted and filtered, and then the residue was washed with water till the filtrate was free of alkali and the filtrations were repeated till the residue gave no colour with water. Treatment with calcium chloride solution and then washing with water was done to neutralized colouring matter in the residue. A small portion of residue material was placed with a drop of glycerine on a clear microscopic slide and mounted with 1% aqueous solution of safranin and covered with cover slip. Observations were made with the light microscope to determine the stomatal complex types, epidermal cells and trichomes present in the tea samples. Photomicrographs were taken with Amscope camera attached to the eye piece of the microscope.

Determination of organic colouring matters in commercial teas

Prussian blue was determined by treating the commercial tea samples with hot sodium hydroxide solution acidulate with acetic acid and ferric chloride. Blue precipitate indicates the presence of Prussian blue. Under microscope, indigo appears as greenish blue. It forms deep solution with addition of sulphuric acid in tea. With addition of sodium hydroxide or any alkaline solution, the cells turn brown, swell up and outline of the granules become visible, indicating the presence of turmeric (Spence, 1976).

RESULTS

Camellia sinensis has hypostomatic leaves condition i.e. having stomata on abaxial surface only with paracytic and anomocytic stomatal complex types, thick-cell wall which is impregnated with waxy material. The occurrences of paracytic stomatal complex are more frequent than anomocytic stomatal complex. Non-glandular unicellular trichomes were also identified. The anticlinal wall pattern was some wort straight in the abaxial surface and shape is rectangular i.e. length and width are unequal and undulated on the adaxial surface (Plates 1).

Table 2 shows the microscopic characteristic features of the six studied commercial samples of green and black teas as described below:

C-tea: Two different types of stomatal complex types were identified on the surface views i.e. paracytic and pericytic, unicellular non-glandular and multicellular glandular trichomes with thick cell wall oval in shape.

L-tea: Paracytic and anomocytic stomatal complex types were observed on surface views of Lipton tea, unicellular non-glandular trichomes and multicellular non- glandular trichomes with thick cell wall oval in shape.

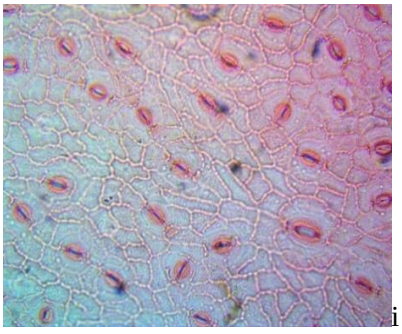
T-tea: Occurrence of three stomatal complex types on surface views i.e. paracytic, polocytic and anisocytic. Unicellular glandular and multicellular non-glandular trichomes with isodiametric, oval shaped thick cell wall.

H-tea: Paracytic and anomocytic stomatal complex was observed on microscopic views with thick cell wall (Table 2).

N-tea: Paracytic, tetracytic and anomocytic stomata types were observed on microscopic views with thick cell wall oval in shape.

A-tea: Only paracytic stomata type was observed with non-glandular trichomes.

In addition to the microscopic examination of the selected commercial tea samples, chemical tests carried out for detection of colouring matters or facing such as indigo, Prussian blue and turmeric showed that there were traces of presences of colouring matters (Table 3).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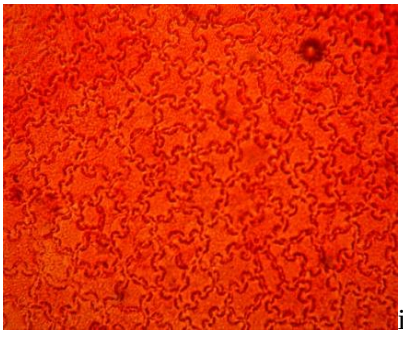
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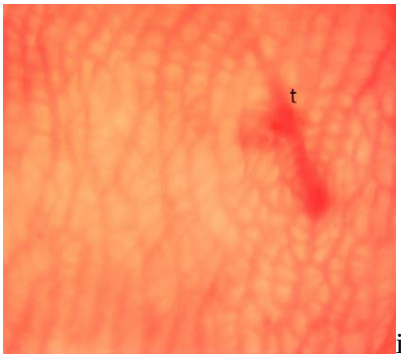

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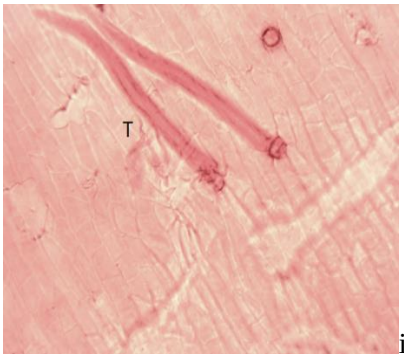

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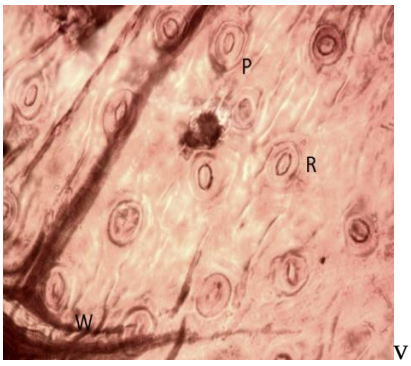

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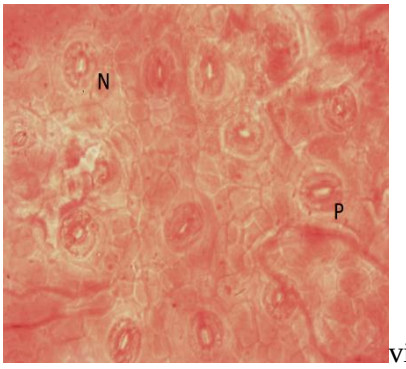

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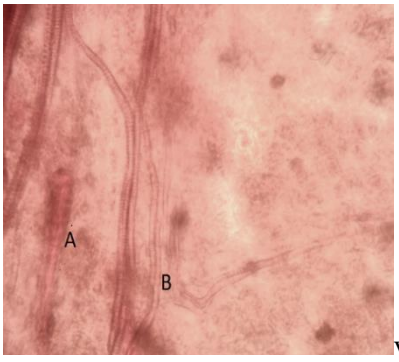

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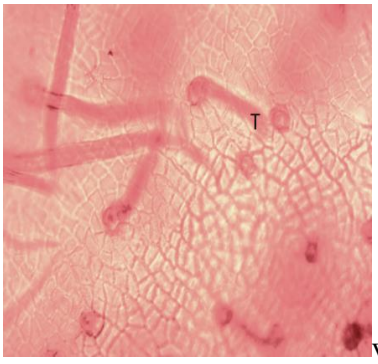

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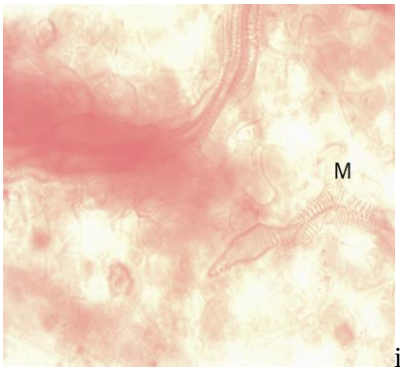

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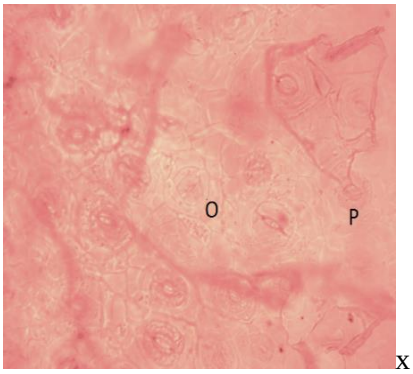

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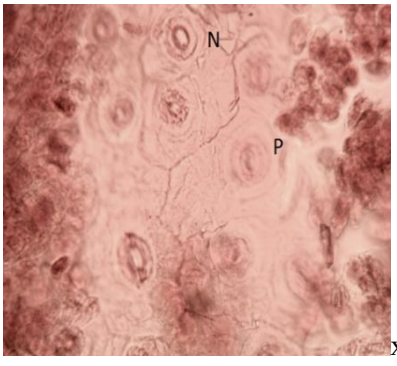

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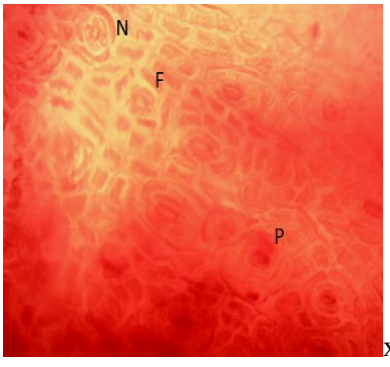

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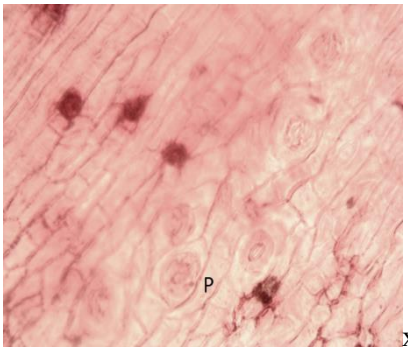

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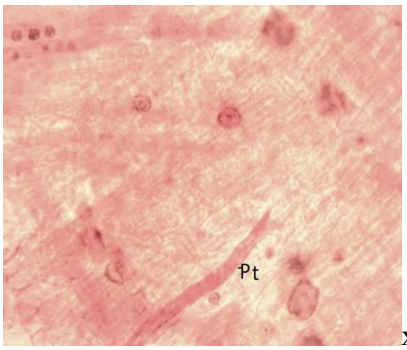

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Plate 1: Surface views of leaf of: *Camellia sinensis* (abaxial [i] and adaxial [ii]) showing anomocytic (a) and paracytic (b) stomatal complex types, unicellular non-glandular trichomes (t) and epidermal cell with unicellular thick cell wall; C-tea showing (iv) unicellular non-glandular trichome (T) with thick cell wall oval in shape, (v) paracytic (P) and pericytic (R) stomata complex types and multicellular non-glandular trichome (W); L-tea showing (vi) paracytic (P) and anomocytic (N) stomata complex, (vii) unicellular non-glandular (A) and multicellular non- glandular trichomes; T-tea showing (viii) unicellular glandular trichomes (T) with thick cell wall, (ix) multicellular non-glandular trichomes (M), (x) Paracytic (P) and anisocytic stomata complex (O); H-tea showing (xi) paracytic and Anomocytic stomata complex; N-tea showing (xii) paracytic (P), teracytic (F) and anomocytic (N) stomatal complex with thick cell wall; A-tea showing (xiii) paracytic (P) stomatal complex and (xiv) unicellular non- glandular trichome (Pt).

DISCUSSION

Anatomical studies apart from special references to systematic position of the taxa can also be used in noting the origin, natural distribution extent of cultivation, and cultivars within species of plants (Lawson, 1967; Onwueme, 1978). Stomata is one of the major characters one looks out for when considering variation in plant and are constant cells within the same taxon. They revealed the differences in species and therefore, important in determining the origin of a particular species. Previous works (Keng, 1962; Ao *et al*., 2002; Yang *et al*., 2003; Ao *et al*., 2007; Yi *et al*., 2019;) have described stomatal patterns in the genus *Camellia*. The variation in the stomata complex type in the tea samples studied in this work proved the relevant of anatomical studies in adulteration of tea samples.

Presence of paracytic and anomocytic stomatal complex types in *C. sinensis* observed in this study was earlier reported by Marcia and Daniel (2006). They observed that anomocytic stomata are found on the abaxial surface of the leaves with unicellular non-glandular trichomes. Also, the report of Erxu *et al*. (2008) that Epidermal cells with walls are scarcely seen on the abaxial epidermis conform with our study.

Meanwhile, some alterations which are anomalous were observed in four of the studied commercial teas. Occurrences of pericytic stomatal complex in C-tea; multicellular glandular trichomes in L-tea; Polocytic and anisocytic stomatal

complex types with multicellular glandular trichomes in T-tea; and occurrence of tetracytic stomatal complex in N-tea which does not correspond to the anatomical structure of the genuine leaf of the *C. sinensis* showed traces of foreign matter anatomically. The work of Ahmad *et al*. (2009) had revealed that leaves and aerial parts of plants such as Chenopodium *ambrosoides* are used to substitute the original *C. sinensis* leaves. They reported that *C. ambrosoides* can be distinguished from *C. sisnensis* by presences of multicellular non-glandular trichomes.

Colouring agents are commonly used in most commercial teas for facing purpose. The work of Spence *et al*. (1976) and Spence (1987) showed that teas are subjected to many forms of sophistication facing and colouring. Both agents may be practised together, since the facing material may be a colouring body, or the colouring itself be a heavy material added to the processed tea. In general, facing is practiced by treating the prepared leaves with the mixture containing Prussian blue, turmeric and indigo.

Evidences of colouring or facing were also detected in fire of the studied teas. This is elucidated in the green teas which were treated with turmeric and black teas were treated with Prussian blue. Prussian blue was detected in L-tea, C-tea and T-teas which showed black colouration with blue patches while turmeric was detected in N-tea and A-teas which turn brown on addition of sodium hydroxide swell up and outline of the granules become visible.

Identification of adulteration depends on a stable, logical, and basically sound system of classification. Fortunately, anatomical studies and chemical tests can be applied to detect any adulterant in commercial tea samples. Anatomically, C-tea, L-tea, N-tea and T-tea, and chemically, C-tea, L-tea, T-tea and A-teas showed traces of adulteration. It is only H-tea and L-tea that possessed both paracytic and anomocytic stomatal complex types, and C-tea possessed unicellular and multicellular non-glandular trichomes as observed in *C. sinensis*, and it was only H-tea with no evidence of treatment with Prussian blue and turmeric.

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Table 1:
List of some commercial teas used as studied materials

Code for the Commercial tea	Manufacturer	Tea type	Place of collection
C-tea	SRI Lanka	Black tea	Oja Oba
L-tea	Unilever	Black tea	Oja Oba
T-tea	Promasidor	Black tea	Oja Tuntun
H-tea	Mambila	Black tea	Oja Oba
N-tea	SRI Lanka	Green tea	Oja Tuntun
A-tea	Alokozgy	Green tea	Oja Tuntun

Table 2:
Anatomical features of *Camellia sinensis* and some commercial tea samples

Plant species and Tea Samples	Stomatal complex type	Trichomes	Epidermal cell shape
Camellia sinensis	Paracytic anomocytic	Unicellular non-glandular	Straight and rectangular in abaxial Undulating in adaxial, with thick cell wall
C-tea	Paracytic, pericytic	Unicellular non-glandular and multicellular glandular	Oval and thick cell wall

Plant species and Tea Samples	Stomatal complex type	Trichomes	Epidermal cell shape
L-tea	Paracytic, anomocytic	Unicellular and multicellular non-glandular	Oval and thick cell wall
T-tea	Paracytic, polocytic, anisocytic	Unicellular glandular and multicellular non-glandular	Isodimetric, oval and thick cell wall
H-tea	Paracytic, anomocytic	Absent	Oval and thick cell wall
N-tea	Paracytic, tetracytic	Absent	Oval and thick cell wall
A-tea	Paracytic	Non-glandular	Oval and thick cell wall

Table 3
Observation of chemical colourings in some commercial teas

Commercial tea samples	Tea types	Observations			Results		
		Prussian blue	Indigo	Turmeric	Prussian blue	Indigo	Turmeric
C-tea	Black tea	Deep blue	Foamy black ppt	-	Present	Absent	-
L-tea	Black tea	Black ppt with patches of blue colour	Foamy black ppt	-	Present	Absent	-
T-tea	Black tea	Black colour with blue patches	Foamy black ppt	-	Present	Absent	-
H-tea	Black tea	Black with yellow patches	Foamy black ppt	-	Absent	Absent	-
N-tea	Green tea	-	-	The cell turns deep brown and swell up	-	-	Present
A-tea	Green tea	-	-	The cell turns deep brown and swell up	-	-	Present