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Table of Contents: Volume 10 Number 4 April 2018

ARTICLES

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Full Length Research Paper

Pharmacognostic evaluation of the leaf of *Microtrichia perotitii* DC. (Asteraceae)

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***Microtrichia perotitii* DC. belongs to the family Asteraceae (Compositeae) and it is a herb found in the West African countries. The aim of this research was to establish pharmacognostic standards for *M. perotitii* through macroscopic, microscopic, chemo-microscopic and physico-chemical investigations. The macroscopic studies revealed the shape of the leaf as small with acute apex, asymmetric base, pubescent surface with long petiole and a serrated margin. Organoleptically, the leaf is green, slightly bitter and pepperish with an unpleasant odour. The microscopic studies of the leaf showed epidermal cells with irregularly thickened walls, numerous anomocytic stomata, and multicellular covering trichomes on both upper and lower epidermis. The transverse section of the leaf showed that it was dorsiventral with separated elements of vascular bundle. The powdered leaf revealed calcium oxalate crystals (prism and rosette), starch (oval) and xylem (spiral). Quantitative-leaf microscopy revealed the leaf constants as palisade ratio (3.2-3.4); stomatal number (258-285); stomatal index (19.5-24.7); vein-islet number (6.0-8.0) and vein-let termination number (8.0-11.0). The physico-chemical constants of the leaf showed moisture content (12.67%), total ash (20.33%), acid –insoluble ash (27.3%), water- soluble ash (81.18%), water extractive values (40.9%) and alcohol extractive values (25.45%). This is the first time the pharmacognostic parameters of the leaf of *M. perotitii* were studied and it will be quite useful for its identification, standardization and inclusion in various pharmacopoeias.**

Key words: *Microtrichia perotitii,* macroscopical, microscopical, physico-chemical, pharmacognostic.

INTRODUCTION

Standardization of herbal medicines (drug) is a process of establishing or prescribing a set of peculiar identities or specific characteristics which are generally unique and of unshared qualities. However, amongst various techniques used in identification of plant drugs, pharmacognostic study is always the most reliable. Basic component are standardization and authentication of natural drugs (Chandaz, 2014). Plant used in traditional medicines are authenticated through morphological, physico-chemical and phytochemical analyses (Chandaz, 2014). One of the major challenges of identification of plant is adulteration which simply means replacing the original plant with another one with the intention of increasing either the weight or potency of the product

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(Rokad et al., 2018). The therapeutic efficacy of any medicinal plant depends generally on the quality and quantity of the chemical constituents it contains (Majumder, 2011; Kumar and Rathinam, 2013). Information about the efficacy of medicinal plants that were passed from generations to generations after sometime could be incorrect, distorted, or even lost and under such circumstances proper scientific evidence must be established through pharmacognostic evaluation (Sumitra, 2014; Evans, 2009). Medicinal plants are the greatest assets to human health and a treasure for discovering new potential compounds with various therapeutic effects. The use of plant drugs locally is becoming increasingly popular especially in modern society because of incidences of adverse effects of synthetic drugs after they have been used. This is a common practice amongst developing countries most especially in Africa where it is their greatest hope (Gireesh et al., 2015; Elujoba et al., 2005).

M. perotitii DC. belongs to the family Asteraceae and is widely distributed in West African countries of Nigeria, Senegal, Mali, Port of Guinea, Sierra Leone, Ivory Coast, Ghana and Dahomey (Hutchinson and Dalziel, 1963). In Nigeria it is found in northern part of the country where it is known as *Maijankai* or *Sawun keke* in Hausa, *Osete* in Igbira and *Shaware pepe* in Yoruba. The herb is an annual plant and is a diffused much branched pubescent varying from 1ft in height. The leaves are obovate, obtuse, cuneately narrowed into petiole, coarsely toothed above ½ - 1½ inch long, ⅓ – 1 inch broad. The petiole of lower leaves is 1inch or more at the upper. Other features are in accordance to some members of the family (Daniel, 1877; Andrews, 1954; Watson and Dallwitz, 1992). Traditionally, *M. perotitii* is used for treating pain related diseases such as toothaches, cuts and burns and rashes in children. Others include, skin disease, rheumatism, diarrhea and jaundice. Earlier phytochemical studies of the leaves revealed the presence of tannins, flavonoids, alkaloids, carbohydrates, cardiac glycosides, saponins, phenolics and terpenoids (Abdullahi et al., 2011). In spite of these medicinal uses of this herb, there are no pharmacognostic reports on the leaves' constants and other parameters as such this study is aimed at providing pharmacognostic standards of the leaves for the first time.

MATERIALS AND METHODS

Collection and preparation of the herb

The herb *M. perotitii* was collected from Rigasa village, Kaduna State, Nigeria in the month of April 2016. The fresh herb was compared with specimen authenticated by Mal. Musa M. of the herbarium unit, Department of Biological sciences, Ahmadu Bello University, Zaria and was given a voucher number 998 for future references. The fresh herb was allowed to dry under the shade for three weeks until all the leaves have fallen-off the stalk. They were reduced to coarse powder with traditional pestle and mortar. Both fresh and the coarse powder were used for the studies.

Macroscopic study

The fresh leaves of *M. perotitii* were subjected to morphological studies in order to evaluate appearance such as surface, dimensions, point of attachment, lamina including composition, inclusions, shape, base, venation, margin and apex as well sensory profile that is colour, odour, texture and taste. The methods described by Brain and Turner (1975), WHO (2007 and Evans (2009) were adopted.

Microscopic study

Both fresh and powdered leaves were studied:

Qualitative examination: For this study, the surface of the leaf of *M. perotitii* was prepared by peeling –up the upper and lower epidermis with sterilized forceps while the transverse section was prepared with a sharp razor blade. Each of the preparations was cleared of opaque materials by boiling them in 70% chloral hydrate solution. Small quantity of the powdered leaves was also prepared by clearing them in 70% chloral hydrate solution after being boiled in a test-tube on a Bunsen flame. Epidermal and other anatomical features were observed from the surfaces and transverse section of the leaf. The standards methods of Brain and Turner (1975), WHO (1984, 2007) and Evans (2009) were adopted.

Chemo-microscopic examination: For this study, the powdered leaves of *M. perotitii* were first cleared of obscured materials by boiling them in 70% chloral hydrate solution. Fragments of the leaves were transferred onto clean microscopic slide with dilute glycerol to prevent dehydration or hardening. Detecting reagents for metabolites such as starch, lignins, cellulose, calcium oxalate crystals, fixed oils and fat, calcium carbonates, mucilages and tannins were applied for their presence in cells inclusions or cell wall. These examinations were carried out according to the methods described by Brain and Turner (1975), WHO (2007) and Evans (2009).

Quantitative leaf examination: For this study, fresh leaf of *M. perotitii* was prepared by peeling–off both the upper and lower epidermis and clearing them by in boiling 70% chloral hydrate solution. The cleared leaf was mounted on clean microscopic slide and covered with cover slip. A set-up camera Lucida at x10 objective (1 small stage micrometer division = 10 µm; calibration factor = 2.7) was set for the determination of stomatal number, stomatal index, vein-islet number, veinlet termination number and palisade ratio. The length and width of stomata and trichomes were measured by calibrating the eyepiece micrometer using stage micrometer as described by Evans (2009).

Physical constant examination: For this study, various physico-chemical parameters were determined for the powdered leaves of *M. perotitii* and these included; moisture content, total ash, acid-insoluble ash, water soluble ash, water soluble extractive values and alcohol extractive values. The evaluation was carried out according to the methods described (WHO, 1998; Evans, 2009; Pratima and Mathad, 2011).

Statistical analysis

The data obtained were expressed as mean ± standard error of

Table 1. Macroscopic and organoleptic characters of the leaf of *Microtrichia perotitii.*

Features		Descriptions
Shape		Small
Dimension (length x breadth)		(3.6- 3.7 mm) × (1.6 -2.1 mm)
Arrangement		Alternate
Petiole		Long
Lamina	I. Composition	Simple
	Ii .venation	Pinnate
	Iii. Margin	Serrated
	Iv. Apex	Acute
	V. Base	Unequal
	Vi. Texture	Brittle
	Vii. Surface	Pubescent
Organoleptic properties:		
	I. Colour	Greenish
	Ii. Odour	Unpleasant
	Iii. Tastes	Slightly bitter and pepperish

mean (SEM), and n represents the number of replicates in a particular experiment.

RESULTS

Macroscopic examination

Macroscopically, the leaf of *M. perotitii* was observed to be small with pubescent surface and alternately arranged along the smooth and soft stalk. Its apex is cute, the base is unequal and the margin is serrated while the venation is pinnate. The dimension of the leaf was 3.6 to 3.7 mm in length and 1.6 to 2.1 in breadth. The leaf when it is fresh is always greenish with unpleasant odour while its taste is slightly bitter and pepperish (stringent) (Figure 1). The summary of the macroscopic and organoleptic observations are given in Table 1.

Microscopic examination

The result of microscopic evaluation of the leaf of *M. perotitii* showed typical characteristics of leaves in terms of taxonomic importance that is the presence of lower and upper epidermis, xylem, phloem, vascular bundles, mesophyll, collenchyma and trichomes. The observed shapes of the cells showed that they appeared elongated at the lower epidermis while they are diagonal at the upper epidermis. The anticlinal walls are wavy at the lower epidermis while they are straight at upper epidermis.

The stomata were anomocytic type and were more frequent at the lower epidermis. Uniseriate multicellular trichomes were identified at both the upper and lower surfaces but were rare at the upper surface (Figures 2 to 6). A summary of the features are summarized in Table 2. From the powdered leaves, fragments of spiral types of xylem vessels, calcium oxalate crystals and starch grains were identified (Figure 6).

Chemo-microscopic evaluation

The chemo-microscopic evaluation of the powdered leaf of *M. perotitii* revealed the presence of cellulose cell wall, lignified cell wall, calcium oxalate crystals, tannins and fat and oils (Table 3).

Quantitative evaluation

The result of quantitative evaluation of the leaf of *M. perotitii* is presented in Table 4.

Physico-chemical examination

The result of the physical constant evaluation is presented in Table 5. The value of moisture content that is loss on drying has indicated the drug cannot easily be deteriorated by fungal or microbial attacks.

DISCUSSION

The pharmacognostic analysis of *M. perotitii* carried-out will help in establishing its botanical identity. The standardization of the herbal medicines is necessary to assure the quality of the drug and will also help in checking and preventing substitution and adulteration of foreign material, that is by mixing or substituting the original drug material with other spurious, substandard, defective, spoiled, useless other parts of the same or different plants (Rokad et al., 2018). It is a common knowledge that plant drugs are used locally for treatment of various disease conditions without recourse to standardization in other to establish the correct identity of the drug (Periyanayagam and Karthikeyi, 2013).

The macroscopic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of drugs. Therefore, the macroscopic characters of *M. perotitii* studied can serve as diagnostic parameters especially its organoleptic characteristics (Singh et al., 2010; Sathis et al., 2011). The organoleptic characteristics of the leaf were unique being bitter and pepperish with unpleasant odour. Microscopic evaluation is one of the simplest and cheapest methods to establish the correct and accurate identity for a plant drug (Patel and Zaveri, 2011). The microscopic evaluation of the leaf

Figure 1. Leaf morphology of *Microtrichia perotitii* ×100.

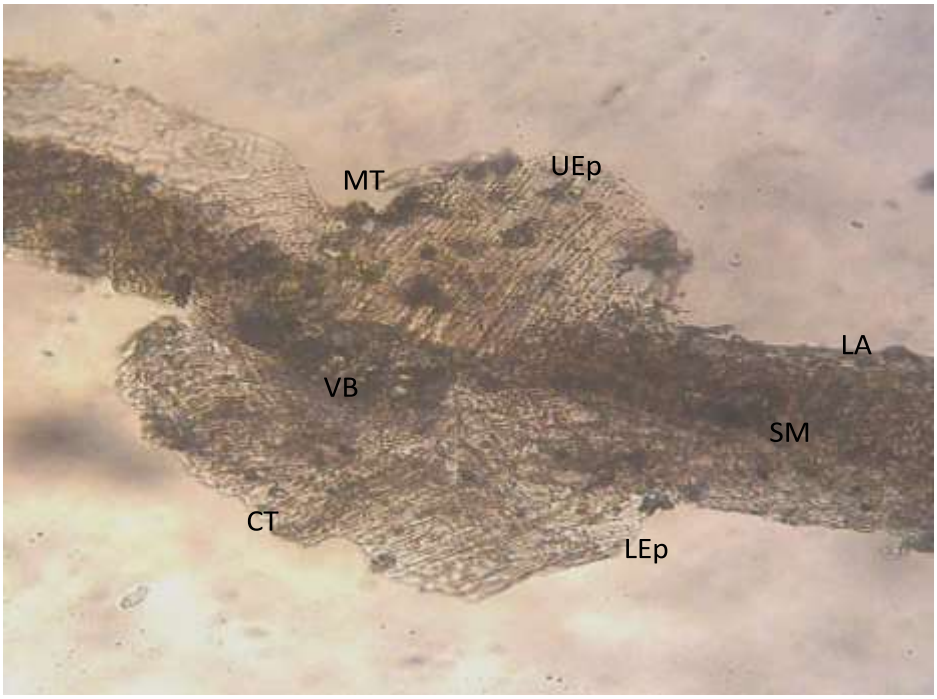

Figure 2. Photomicrograph of the transverse section of the midrib of the leaf of *Microtrichia perotitii* showing multicellular trichomes (MT), upper epidermi (UEp), covering trichomes (CT), spongy mesophyll (SM), lower epidermis (LEp), lamina (LA) and vascular bundles (VB)×100.

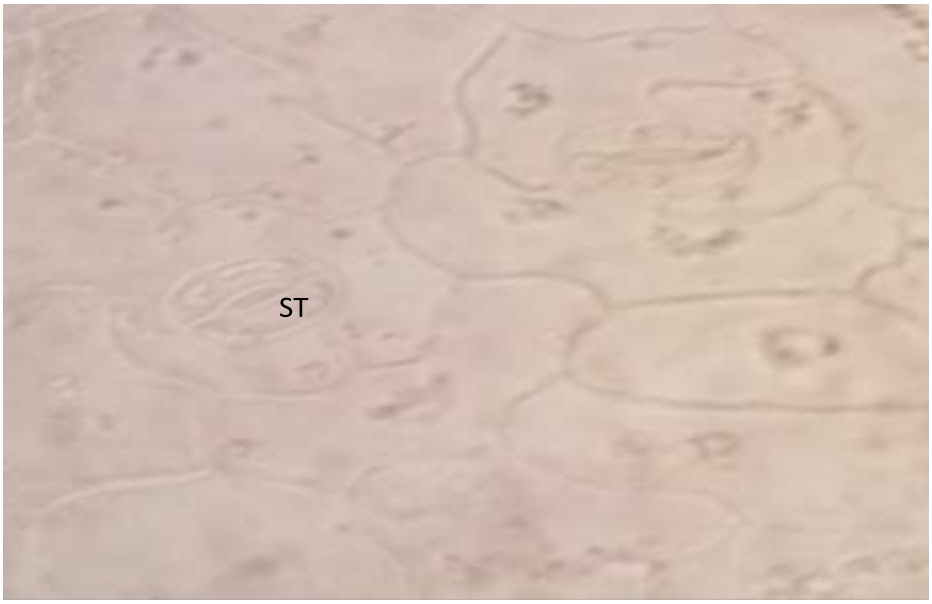

Figure 3. Photomicrograph of the surface preparation of upper epidermis of the leaf of *Microtrichia perotitii* showing anomocytic stomata (ST) ×100.

Figure 4. Photomicrograph of the surface preparation of lower epidermis of the leaf of *Microtrichia perotitii* showing anomocytic stomata (ST) and wavy anticlinal cell wall (WC) ×100.

of *M. perotitii* showed that the leaf has anomocytic stomata occurring at both adaxial and abaxial epidermis thus indicating efficient gaseous exchange for photosynthesis and loss of water (Shaukat et al., 2010). Multicellular trichomes also appeared on both surfaces of the leaves although not so frequent at the upper surface. The presence and types of trichomes are useful diagnostic features and they contribute to

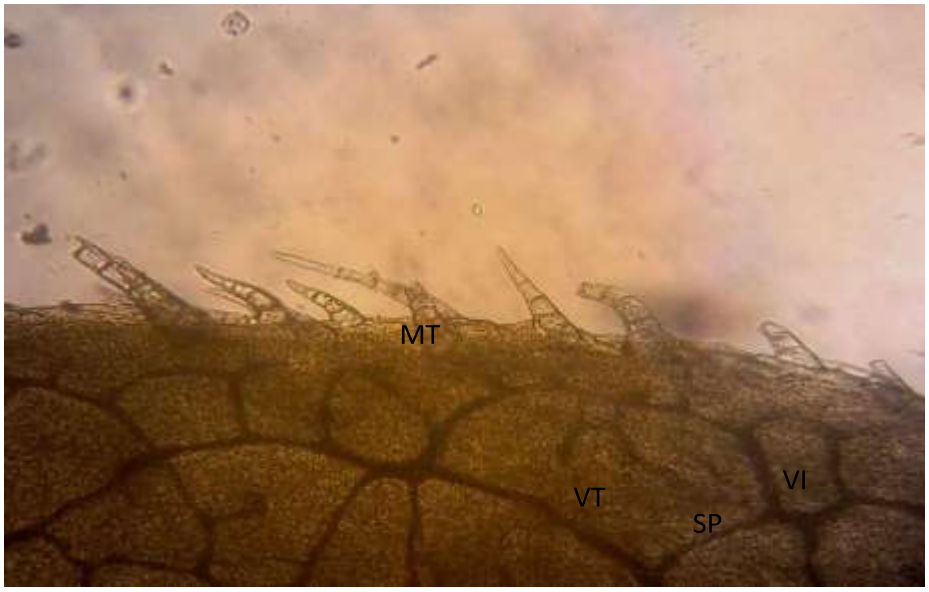

Figure 5. Photomicrograph of lower epidermis of the leaves of *Microtrichia perotitii* showing multicellular trichomes (MT), veinislet (VI), vein termination (VT) and spongy mesophyll (SP) x 100.

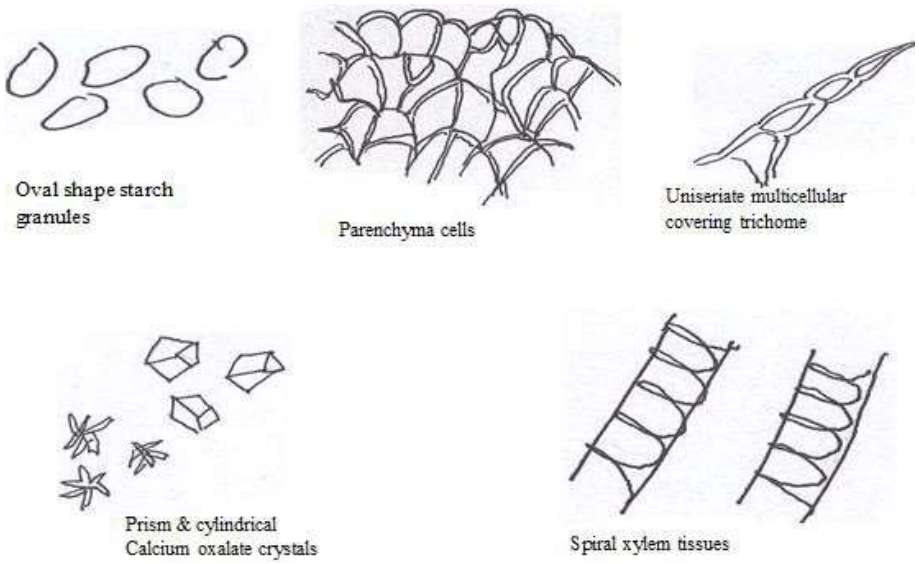

Figure 6. Some identified microscopic diagnostic features from the powdered leaves of *Microtrichia perotitii* x 400.

plant resistance against herbivory and reduction of the rate of transpiration in the plants (Ahlam and Bouran, 2011; Priyanka et al., 2011a, b). The transverse section of the leaf indicated that it is dorsiventral and showed the appearance of a typical leaf structure. Calcium oxalate crystals were observed and they may be involved in the dispersing of light to the chloroplasts in the photosynthetic parenchyma cells of the leaves. The crystals were thought to have a physiological role sequestering excess calcium within plant cells (Popescu et al., 2010). Starch granules were observed and appeared in oval shape. Although the starch is the main form in which plants store carbon, they are sometimes converted into sugar by amyloplats when the plant needs energy (Alison, 2010). Chemo- microscopic evaluation of the leaf showed proteins, starch, cellulose and tannins including calcium oxalate crystals and lignins which are indications of the presence of

Table 2. Epidermal characters and their descriptions.

Features	Characters	
	Lower epidermis	Upper epidermis
i. **Cells:** Shapes	Elongated	Polygonal
Anticlinal wall	Wavy	Straight
Thickening	Smooth and cellulose	Smooth and cellulose
Papillae	Absent	Absent
Cuticle	Present	Present
ii. **Stomata:** Type	Anomocytic	Anomocytic
Frequency	Numerous	Frequent
Size (l,b,µm)	27×16.2	27×13.5
iii.**Trichomes:** Type	Uniseriate multicellular	Uniseriate multicellular
Frequency	Frequent	Rare
Size(h,bµm)	135×21.6	132×21.6

l = length; base= base; h = height.

Table 3. Chemo-microscopy result of the leaf of *Microtrichia perotitii.*

Constituents	Mounting reagent	Result/observation	Inference
Starch	Iodine solution	Blue-black colouration appeared on some grains within the chloroplasts and powdered leaves	Present
Lignin	Phloroglucinol and conc. HCl	No red colouration observed on the walls of some lignified collenchymas.	Present
Cellulose	Chloro-zinc iodine solution	Blue colouration observed on walls of epidermal cells	Present
Calciumoxalate	Chloralhydrate solution	brightly coloured crystals observed which later dissolved in conc. HCl and disappeared in the collenchymas cells	Present
Oils and fats /warming	Sudan iv solution	Reddish colouration was observed in some parenchyma cells and was distinct	Present
Mucilages	Ruthenium red solution	Dark solution observed at epidermis and vascular tissues	Absent
Tannins	5% ferric chloride solution	Greenish black colouration was observed in some parenchyma cells	Present

Table 4. Quantitative leaf microscopic features of *Microtrichia perotitii.*

S/N	Features	Characteristics
i.	Palisade ratio	3.2 -3.3* - 3.4
ii.	Stomata number	258-271.5* -285(adaxial epidermis) 289-299.5*-310(abaxial epidermis)
iii.	Stomatal index (%)	19.5- 22.1*-24.7 (adaxial epidermis) 29.3 -31.6*- 34.5 (abaxial epidermis)
iv.	Vein-islet number	6.0 - 7.2* - 8.0
v.	Veinlet termination number	8.0- 9.6* -11.0

*n = 5.

alkaloids, flavonoids and glycosides in the leaves (Prabhu et al., 2009).

Quantitative microscopic evaluation of the leaf of *M. perotitii* has provided values for palisade ratio, stomatal number, epidermal layer, stomatal indices, vein islet number and veinlet termination number. These information set genuity and standard for the herb as well as distinguishing it from co-generic species that may be closely related and cannot easily be characterized by general microscopy (Veeranjaneyulu and Rama, 1984).

The physico-chemical evaluation of the leaves of *M.*

Table 5. Physicochemical constants from the leaves powder of *Microtrichia perotitii.*

Parameter	Mean values (%)
Moisture content	12.67±0.62
Total Ash	20.33±0.20
Acid-insoluble Ash	2.73±0.13
Water-soluble Ash	8.18±0.30
Water-soluble extractives	40.9±1.73
Alcohol- soluble extractives	25.45±1.24

n = 3.

perotitii could serve as a significant role in standardization and quality control by means of purity, stability and phytochemical composition of the herb (Bharat and Parabia, 2010). The moisture content for *M. perotitii* calculated by loss was 12.67% which is less than 14% standard requirement for crude drugs. This is an indication that the powder of this herb can be stored for a longer period of time without spoilage (Ahmad et al., 2012; Kadam et al., 2012; Mubo et al., 2014). The total ash value for *M. perotitii* lies within fair limits and thus signified its quality and purity and gives idea about the total inorganic content in it (Ugur and Selima, 2011; Woratouch et al., 2011). The acid- insoluble ash value of 2.73±0.13% obtained for the leaves is an indication that the herb was in good physiological condition thus contained less extraneous matter in the form of contamination with silicious materials (earth and sand) and provides information about non-physiological ash produced due to adherence of inorganic dirt and dust to the crude drug. The water-soluble ash was 8.18±0.30% and this parameter is used to detect the presence of materials exhausted by water (Schoffstall, 2000; Singh and Sharma, 2010; Adedapo et al., 2011; Pratima and Pratima, 2011; Ahamad et al., 2012; Kamalakannan et al., 2012 ; Kunle et al., 2012; Veena and Pracheta, 2013; Sangram et al., 2015).

Extractive values of plants give useful estimation of chemical constituents in the drug as well as a measure of the stability of phytocompounds in the plant drug in a given solvent. The value for the water soluble extractive for the leaves of *M. perotitii* were much higher than that of the alcohol thus indicating higher polarity and more penetration of the water molecules into the cellular membrane in order to extract all inter-cellular components of the plant material. The leaves therefore, exhibited more amount of water soluble components compared to alcohol extract and may provide estimation of specific constituents soluble in a particular solvent (Periyanayagam et al., 2013; Vipin et al., 2015).

All the results obtained for the pharmacognostic evaluation of the leaves of *M. perotitii* were compared to those of some members of Asteraceae in order to maintain a standard since this is the first report about the leaves in the literature.

Conclusion

The pharmacognostic study on the leaves of *M. perotitii* is being reported for the first time in literature. The findings will be useful in the identification of the herb, detection of adulterants and its monograph. It will also provide avenue for further studies on microscopic evaluation of the leaves.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of Ethiopian high land green tea (*Camellia sinensis*) leaf extract on highly active anti-retroviral therapy induced dyslipidemia in Albino Wistar rats

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Highly active antiretroviral therapy (HAART) is considered toxic and has other life-threatening side effects including dyslipidemia. There is no research report on the health effects of Ethiopian highland green tea. Previous work data from elsewhere suggest that lipid abnormalities are associated with cardiovascular morbidity and mortality. The aim of this study was to investigate the possible protective effect of green tea (*Ocimum gratissimum)* hydro-ethanolic leaf extract on highly active antiretroviral therapy induced dyslipidemia in albino Wistar rats. Thirty rats of age 10 to 12 weeks and similar weights were selected and divided in to 5 groups of six rats each. Group-I (normal control group) were given distilled water, Group II were given HAART only, Groups III, IV and V were given antiretroviral therapy and 100, 200 and 400 mg/kg of extract, respectively for sixty days. The dissolved crude extracts of different doses were given to rats using oral gavage. On experiment day, the rats were fasted overnight, sacrificed by cervical dislocation and blood was taken by cardiac puncture for lipid profile investigation. Lipid profile was measured spectrophotometrically using standard kits and procedures. Elevated levels of serum total cholesterol, triacylglycerol, low density lipoprotein cholesterol and high density lipoprotein cholesterol were observed in highly active antiretroviral therapy treated group. The rats that received HAART+400 mg of *O. gratissimum* showed a significant decrement of serum total cholesterol, triglycerides, and low density lipoprotein cholesterol ($p<0.05$) with no alteration of high density lipoprotein cholesterol. The green tea leaf extract with a dose of 400 mg/kg has a good protective effect against HAART induced dyslipidemia which might be due to its antioxidant property.

Key words: Highly active anti-retroviral therapy, green tea leaf extract, dyslipidemia, rats.

INTRODUCTION

Highly active antiretroviral therapy (HAART) regimens are currently in use and have been found effective in increasing life expectancy and immune status of HIV positive patients in the world since 1996 (Rouleau et al.,

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2011). These HAART regimens typically include a combination of at least three drugs, such as different association of protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors and nucleoside/tide reverse transcriptase inhibitors (Gsponer et al., 2012). Thus, the introduction of HAART has led to a marked reduction in AIDS-related morbidity and mortality, because of the diversity of these drugs and their interference at different sites in the life cycle of the virus and disruption of the progress of viral proliferation in the body of the patient aggressively (Bhaskaran et al., 2008).

However, besides the substantial benefits that result from the use of HAART regimens, laboratory and clinical experience have shown that they can induce considerable side effects on lipid metabolism which is characterized by lipodystrophy, central adiposity, dyslipidemia, increased risk of cardiovascular diseases and atherosclerosis (Sprinz et al., 2010). HAART associated dyslipidemia which is characterized by hypertriglyceridemia, hypercholesterolemia, and decreased serum levels of HDL-c either accompanied or not by increased levels of LDL-c that also involves hormonal and genetic predisposition is one of the complex lipid metabolism derangements (Fisher et al., 2006). HAART also affects the hydrolysis of triacylglycerol rich lipoproteins and tissue lipase, which disrupts normal postprandial free fatty acid and lipoprotein catabolism and interferes with peripheral fatty acid trapping. The NRTI-based HAART, zidovudine, stavudine or lamivudine has become associated with the occurrence of dyslipidemia; however, lipid metabolism disorders are most evident in individuals who are using PIs (Abebe et al., 2014). Protease inhibitors promote dyslipidemia (hypertriglyceridemia, increase in total cholesterol and LDL-c and decrease in HDL-c) even in short-term studies (≤4 weeks) with RTV (Purnell et al., 2000) and LPV/RTV (Lee et al., 2005) in HIV-seronegative men. Protease inhibitors stimulate hepatic lipid synthesis by maintaining the nuclear activity of the sterol regulatory element binding protein (SREBP) (Tran et al., 2003). PIs bind to the glucose transporter (GLUT 4) and prevent glucose transport to the adipocytes and muscle cells but have no effect on liver (Flint et al., 2005). There is also no concrete evidence that PIs affect the transport of fatty acids by fatty acid transporter proteins. Instead, PIs affect hepatocyte metabolism by extending the activity of transcription factors involved in regulating lipid synthesis (Parker et al., 2005).

Camelia genus is an evergreen shrub of the Theaceae family native to Japan, China, India, Southeastern Asia, with dark green shiny leaves and white flowers that is used for preparation of the second largely consumed drink in the world. *Camelia* genus contains caffeine, theophylline, and theobromine, glutamide derivative theanine and also contains many nutritional components, such as vitamin E, vitamin C, fluoride, and potassium. Chacko et al. (2010) revealed the health benefits of consuming green tea which includes the prevention of cancer and cardiovascular diseases, the anti-inflammatory, antiarthritic, antibacterial, antiangiogenic, antioxidative, antiviral, neuroprotective, and cholesterol-lowering effects associated mainly with its antioxidant properties that is attributed to its high content of polyphenols. Most of these polyphenols in tea are flavanols commonly called catechins (Balentine et al., 1997). The main catechins in green tea are epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). These catechins account for 30 to 42% of the dry weight of green tea and EGCG accounts for 50 to 80% of the total catechins (Arshad et al., 2015). It was reported that radical-scavenging ability of EGCG was higher than that of other catechins due to the presence of a gallate moiety in the C ring (Xu et al., 2004). The potency of this property varies from country to country and even place to place in the same country because the content of its active constituents are affected by the type of soil, climate, and season of collection and age of the plant (Rani et al., 2014; Govarthanan et al., 2015).

The aim of the present study was to explore antilipidemic roles of hydroethanolic extract of *Camelia* genus in HAART-induced dyslipidemia. To date no such work has been done on the Ethiopian highland green tea and to our knowledge there is no any report on science journals.

MATERIALS AND METHODS

Plant and extraction

The packed green tea was purchased from Ethio Agri-CEFT private limited company in Ethiopia. The coarse powder of 918 g of green tea (*Camellia sinensis*) was macerated in 80% methanol (W/V) with a ratio of 1:10 for three days (72 h) by mechanical shaking at room temperature. The extract was first filtered using filtering cloth followed by Whatman filter paper No. 1 and then the filtrate was dried by rotary evaporator. Then the filtrate was evaporated in thermostatic oven at 40°C to remove the remaining methanol. The final gummy green tea leaf extract (GTE) was lyophilized, weighed, put in tight glass containers and kept in a refrigerator.

In-vitro antioxidant activity determination

Rapid screening of antioxidant activity of green leaf extract was done by dot-blot staining using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity following the method of Soler-Rivas et al. (2000).

Photometric assay of DPPH scavenging activity

The DPPH scavenging activity of the extract of green tea was measured according to the method of Gyamfi et al. (1999) photometrically.

Experimental animals

Thirty adult female albino rats weighing about 200 to 250 g of age 10 to 12 weeks were obtained from Pharmacology Department,

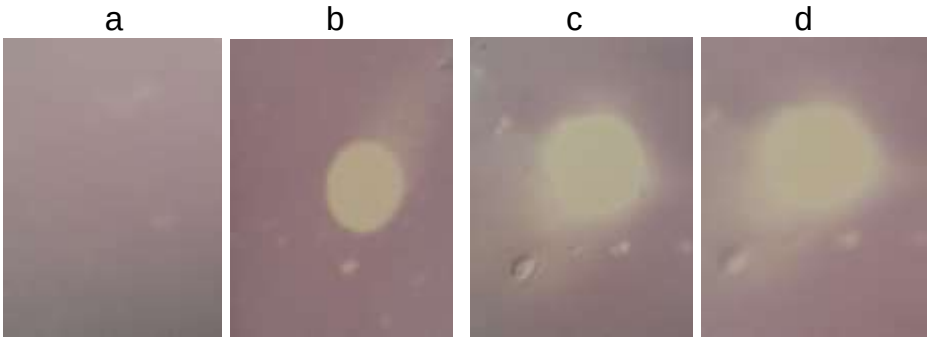

Figure 1. Qualitative *In-vitro* antioxidant activity test of 80% hydro-methanolic green tea leaf extract (GTE). (a) TLC plate which is immersed in 0.1 mM DPPH, (b) 2 µL of 100 mg/100 ml of GTE, (c) 2 µL of 200 mg/100 ml of GTE, (d) 2 µL of 400 mg/100 ml of GTE.

School of Medicine, College of Health Sciences, Addis Ababa University and housed in polypropylene cages and maintained at standard laboratory condition. They were provided with drinking water and standard pellet rat diet supplied by Kality Animal Nutrition Production Ltd., Addis Ababa Ethiopia *ad libitum.*

HAART dose extrapolation to experimental animals

The human dose of HAART drug was extrapolated to rats by the following formula as developed by Chen (2016): Dose for rats = (X mg/kg × 70 kg × 0.018) / 0.2 kg = 6.3X mg/kg, where X = the effective dose for man; 70 kg = the average standard weight of adult human; 0.018 = ratio of the equivalent dose between man and rat based on body surface area; 0.2 kg = the standard weight of a rat. The extrapolated HAART dose was given for rats for 60 days. The HAART given during the study period were in combination of ZDV/3TC+LPV/RTV with adult dose of 300/150 mg Po bid and 200/50 mg 2 tablets Po bid respectively. Then dose for rats were extrapolated as follows: ZDV/3TC (6.3 × 900/70 = 81 mg/kg) and LPV/RTV (250 × 4= 1000 mg which is normal adult dose/day). Hence, dose for rats was calculated as 6.3 × 1000/70 = 90 mg/kg per day.

Ethical approval

This study was conducted after experimental protocols approved by the departmental research and ethical review committee (DRERC), meeting number DRERC 03/15, and by protocol number MSc Thesis 05/15, on 04 September, 2015. All rules applying to animal handling, safety and care were properly followed.

Animal grouping and drug dose

The following are the groups and drug dose: Group-I normal control, given distilled water only; Group-II positive control, given HAART drugs only; Group-III, given HAART drugs + 100 mg/kg of green tea extract/per day/60 days; Group-IV, given HAART drugs + 200 mg/kg of green tea extract/per day/60 days; Group-V, given HAART drugs + 400 mg/kg of green tea extract/per day/60 days.

Blood sample collection and analyses

At the end of the experimental day the rats were fasted overnight and sacrificed by cervical dislocation after anesthesia using diethyl ether. Then blood was collected from each rat by cardiac puncture. Serum lipid profiles, that is, total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL) were measured by using autoanalyzer machine (Humastar300SR, Germany). The protocols given by the manufacturer were used during sample analyses.

RESULTS

The amount of crude extract which was obtained from 918 g of *C. sinensis* leaf coarse powder was 146 g. Therefore, the percentage yield of this extraction by using 80% methanol was calculated and given as:

%Yield = (146/918) × 100 = 15.9% (w/w)

To observe the effect of GTE (*C. sinensis*) against HAART induced dyslipidemia in albino Wistar rats, the *in-vitro* antioxidant activity of GTE was studied qualitatively and quantitatively and lipid derangements were biochemically investigated in all experimental groups and presented in table and figures.

As shown in Figure 1, the diameter of reduced zone increased in direct proportion to increase in extract concentration used. The yellowish pale color shows a reduced zone that masks the color of DPPH and the unreduced region or the region where the antioxidant cannot reach remains violet, which is the color of DPPH. The development of this pale yellowish color shows a reduction of DPPH (violet color) to DPPH-H, consistent with the postulate of Muthusamy et al. (2015) and work of Ebrahimzadeh et al. (2009) which is believed to be due to the transfer of electrons from the reducing agent (antioxidant) to DPPH.

Quantitatively, the IC_{50} (inhibitory concentration 50, the concentration of the extract that decrease the absorbance of DPPH by half) of crude GTE was estimated as shown in Figure 2 which was ~0.16 mg/ml, whereas that of ascorbic acid is ~0.08 mg/ml. This result shows that the crude green tea extract has a closer antioxidant activity with ascorbic acid and also both ascorbic acid and GTE

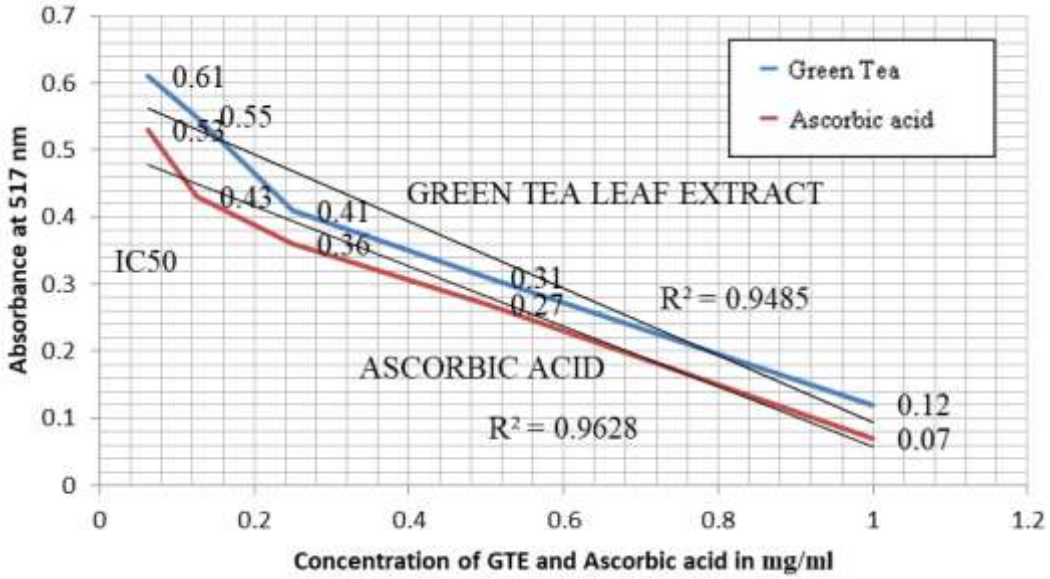

Figure 2. Curve of GTE and ascorbic acid concentration versus absorbance at 517 nm.

Table 1. Effect of green tea leaf extract (*Camellia sinensis*) on serum lipid profile.

Group	TC (mg/dl)	p	TG (mg/dl)	P	HDL-c (mg/dl)	P	LDL-c (mg/dl)	P
I	41±8.07	0.001	47.5±8.59	0.01	35.07±7.01	0.45	7.3±2.16	0.021
II	66.2±12.9	-	69.3±14.32	-	27.67±9.02	-	14.7±5.89	-
III	60±12. 1	0.769	51.7±3.92	0.05	27.17±7.14	1.00	9±3.58	0.746
IV	54.5±3.5	0.208	49.5±13.28	0.022	28.17±7.63	1.00	11±3.52	0.474
V	47.5±4.9	0.013	48.8±8.18	0.017	33.33±6.08	1.00	8±2.28	0.041

P-value is given as compared to group-II, all data are expressed as mean± STD (standard deviation), n=6.

showed a decrease in the absorbance of DPPH in dose dependent manner, that is, their inhibitory effect increased as their concentration increased. The absorbance of 0.008% DPPH was 0.98 which was used as a control and the absorbance of methanol (reference blank) was 0.0017 at 517 nm.

As indicated in Table 1, the HAART-treated rats showed a significant increase in their serum level of TC, TG and LDL-c. However, the serum level of HDL-c showed numerical decrement with no statistical significance. In the GTE treated groups, the serum level of TC, TG and LDL-c decreased but statistically significant reduction was observed in group-V rats which were treated with the highest dose, that is, 400 mg/kg of GTE.

However, serum level of TG was significantly reduced for all doses of GTE. HDL-c also showed a slight increment in all GTE treated groups but it was not statistically significant. Though dyslipidemia is seen sometimes in individuals who are not taking HAART, it is usually associated with HAART intake (Shafran et al., 2005; Chantry et al., 2008; Tassiopoulos et al., 2008). This study showed that, HAART treated group and HAART plus the lowest dose (100 mg/kg) of GTE treated group (Group-III) showed a significant increase ($p<0.05$) in the serum level of TC as compared to normal control group. But, group-V rats which were treated with 400 mg/kg and HAART showed a significant decrease in the serum level of TC as compared to group-II. Group-II also showed a significant increment ($p<0.05$) in the serum level of TG and LDL-c as compared to normal control group (G-I). Group-V rats showed a significantly low ($p<0.05$) serum level of TG and LDL-c.

DISCUSSION

HAART leads to lipodystrophy which is one or more of several metabolic abnormalities typically associated with dyslipidemia; usually due to elevated TC, TGs, LDL-C and low levels of HDL-C (Feeney and Mallon, 2011). It has been reported, that protease inhibitors treatment did

not alter sterol regulatory element binding protein (SREBP) mRNA levels in lipidemic tissues, but promotes the accumulation of more activated SREBP in the nucleus which results in the constitutive induction of lipid biosynthesis through increased expression of lipogenic enzymes such as fatty acid synthase, 4-hydroxymethylglutaryl CoA reductase, acetyl CoA carboxylase and ATP-citrate lyase that cause increased lipogensis in liver. In addition, it also slows down the intracellular degradation of APo-B-100, prompting an overproduction of very low density lipoprotein (VLDL) particles. Antiretroviral drugs may also inhibit the LDL receptor-related protein reducing the clearance of VLDL from circulation (Riddle et al., 2001). Lipoatrophy caused by nucleoside analogues has been proposed to result from inhibition of mitochondrial DNA polymerase gamma within subcutaneous adipocytes. Dideoxinucleosides have a high potential for mitochondrial toxicity, which causes defective beta-oxidation of free fatty acids. Andrea et al. (2010) investigated the effects of HAART on mice and reported that these drugs caused dyslipidemia which contributes to the development of cardiovascular diseases. Findings of the present investigation were in line with those observation, which showed that HAART administration in rats resulted in elevated levels of TC, TG and LDL-C and drastic decrease in HDL-C as compared to normal control.

Green tea as a beverage and as traditional medicine originated and is widely used in Far East countries like China and Japan, and Western countries. It is believed to have healing effect for different health ailments like obesity, diabetes and cancer. This plant is becoming the front line traditional medicinal plant because of its high concentration of polyphenols known as catechins which are unaltered and well preserved in green tea. Catechins in green tea are one of the strongest antioxidants. This antioxidant effect of green tea in turn renders it to prevent a number of metabolic derangements (Zaveri, 2006). The catechins, particularly, EGCG of the green tea inhibits the intestinal absorption of dietary lipids; by interfering with emulsification, digestion, and micellar solubilization of lipids, which results in decreased absorption of TG, cholesterol, and other lipophilic compounds such as a-tocopherol (Loest et al., 2002; Wang et al., 2006a, b; Koo and Noh, 2007). A possible mechanism for the decreased cholesterol may be due to the up regulating potential of GTE on LDL receptor gene which in turn increases the uptake of LDL-C from blood circulation (Bursill et al., 2007). The decrease in serum TG might be due to the suppressing effect of green tea on expression of stearoyl-CoA desaturase (SCD-1) gene which determine hepatic triglycerides synthesis by involving in the biosynthesis of oleate and palmitoleate which are the main monounsaturated fatty acids of triacylglycerol (Rabia et al., 2015). The serum level of HDLc, which is also known as good cholesterol, in HAART only treated group showed a minimal and statistically insignificant decrease as compared to group-I. But, GTE treated groups showed a slight increment though it was insignificant change as compared to group-II. The slight HDL increase is beneficial in that it promotes the reverse cholesterol transport, which avoids cholesterol accumulation and its pathologies.

When the counter effect of various doses of GTE on the serum level of HDL-c of experimental rats is seen, the minimum dose of GTE (100 mg/kg) did not show any change in their serum level of HDL-c in group-III, but groups-IV and V rats showed a relative increment than group-II though not statistically significant. This finding is in agreement with a previous study done by Muramatso et al. (1986) on rats given catechins, where they observed normalization of serum level of TC without significant change in the serum level of HDL-c. The administration of GTE in ovariectomized rats considerably down regulates the hepatic expression of SREBP-1c and its target genes such as FAS and SCD1, and the genes that regulate hepatic cholesterol synthesis (HMGR) and efflux (ABCA1) (Shrestha et al., 2009). This finding suggests that GTE may not alter the expression of genes involved in intestinal lipid uptake and chylomicron assembly. Thus, based on the information available, the TG-lowering effect of GT in plasma and liver may be mediated partly via the suppression of lipogenesis and inhibition of luminal hydrolysis and micellar transfer of lipids to the enterocytes. All these biochemical mechanisms take into account the antidyslipidemic properties of green tea.

Conclusion

Green tea leaf extract has anti-dyslipidemic effect and preventive effect on development of nonalcoholic fatty liver disease satisfactorily and the response is profoundly effective with increasing dosage of GTE administration to rats. It also reverses the dyslipidemia induced by HAART. This indicates that HIV patients who are on HAART treatment will be beneficiary if they take green tea parallel with their antiretroviral treatment. A weight decrement was observed (data not shown) in the GTE treated group indicating that, through protection of dyslipidemia, GTE seems to avoid central obesity and abnormal accumulation of lipids in the adipose tissue.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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