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Effect of Drying Temperature on the Phytochemical and Proximate composition of *Acalypha wilkesiana* Leaves

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ABSTRACT

This work investigated the effects of drying temperature on the phytochemical and proximate composition of *Acalypha wilkesiana* leaves. The leaves were dried at 50 ºC and 80 ºC. Proximate and phytochemical compositions of the dried *Acalypha wilkesiana* leaves were evaluated. The result of the phytochemical screening of the extract revealed the presence of all phytochemicals tested. The quantitative phytochemical composition of the *Acalypha wilkisiena* results for 80 ºC - 50 ºC were 31.33-58.77 mg GAE/g, 18.06-23.39 mg QE/g, 12.04-12.51 %, 44.58-45.59 mg/g, and 5.51-5.68 % for phenolic, flavonoid, saponin, tannin, and alkaloids respectively. The phytochemical concentrations of sample A dried at 50 ºC were significantly higher than sample B dried at 80 ºC. The mean value of fat, protein and carbohydrate for sample B were found to be higher than sample A. Meanwhile sample A had more concentration of ash than sample B. The study has shown that drying of *Acalypha wilkesiana* leaves at different temperature has effect on its phytochemical and nutritional composition.

Keywords: *Acalipha wilkesiana,* temperature, phytochemical, proximate

INTRODUCTION

Medicinal plants are plants which are used for therapeutic purposes and have enjoyed great approval in the treatment of various diseases for many centuries [1]. Medicinal plants contain biologically-active chemical substances(phytochemicals) such as saponins, tannins, essential oils, flavonoids, alkaloids, and other compounds which have preventive or curative properties [2].

These complex chemical substances generally occur as secondary plant metabolites in these plants and are useful to humanity [3]. The various human activities and lifestyle, both personal and industrial, have led to incidences of cancer, diabetes, and heart-related diseases. This has not only affected the Nigeria public health but now a global issue. In addition, the available drugs and antibiotics appear to be ineffective due to the surge of multidrug resistant bacteria. Consequently, researchers are focused on medicinal plants to handle these various threats to health. Examples of medicinal plants that are used in the management of different health conditions are *Vernonia amygdalina* (bitter leaf), *Ocimum gratissimum* (scent leaf), and *Acalypha wilkesiana* (copper leaf).

Acalypha wilkesiana is an ornamental plant common in most gardens and surroundings in Nigeria. *A.wilkesiana* has antioxidant and cytoprotective properties [4]. They are traditionally used in the treatment and/or management of diverse ailments such as diabetes, jaundice, hypertension, fever, liver inflammation, schistosomiasis, dysentery, respiratory problems including bronchitis, asthma and pneumonia as well as skin conditions such as scabies, eczema and mycoses [5]. Antioxidants have been of interest to health professionals due to the protective effect against degenerative diseases caused by reactive oxygen species (ROS), reactive nitrogen species (RNS) [6]. Epidemiological studies have demonstrated that there is a positive relationship between intake of antioxidant rich diets and lower incidence of degenerative diseases such as cancer, heart disease, inflammation, arthritis, immune system decline [7].

Literature survey has revealed that there is scarcity of information on the effect of drying temperature on the phytochemical and proximate analysis of *Acalypha wilkesiana* leaves. Therefore, the aim of this research is to investigate the effect of drying temperature on the phytochemical and proximate composition of *Acalypha wilkesiana* Leaves.

MATERIAL AND METHOD

Collection of Plant Samples

Matured fresh leaves of *A. wilkesiana* were collected by hand-plucking from parent plants at different locations within Babcock University, Ilishan-Remo,Ogun State, Nigeria.

Preparation and Extraction of Plant Materials

The extraction of plant materials was done by the modified method of Adaramola et al [8]. The freshly collected leaves of *A. wilkesiana* were thoroughly washed with tapwater followed by

distilled water. Each leaves sample was divided into two portions. One portion was dried at 50 ºC and the other portion was dried at 80 ºC in the oven. It was allowed to cool and then pulverized with the use of a USHA MG 3473 laboratory grinder. Extraction of the leaves was carried out by maceration in 70% ethanol with sample to solvent ratio of 1: 8. The mixtures were vigorously shaken and allowed to stand for 48 h at room temperature. The mixture was thereafter filtered with a Whatman No. 1 filter paper and the residue was macerated again in equal volume of ethanol for 24 h in order to obtain more quantity of the extract. About 30 mL each of the mixture were taking for the qualitative determination of phytochemicals. The remaining mixtures were combined and then evaporated to dryness. This was done under reduced pressure at about 40 ºC with the use of Eyela N-1001 vacuum rotary evaporator.

Phytochemical Screening

Test for Terpenoids

A volume of 5 ml of the plant extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added to form a layer. A reddish brown coloration of the interface was formed to show the presence of terpenoids [9, 10]

Test for Steroids and Phytosterols

Exactly 2 ml of acetic anhydride was added to 0.5 ml of the plant extract of each sample with 2 ml of H_2SO_4. The colour change from violet to blue green in the sample indicated the presence of steroids and sterols [9].

Test for Tannins

Exactly0.5 ml of the plant extract was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black coloration [10]. Blue colour indicated the presence of Gallic tannins and green black colour indicated presence of Catecholic tannins [9, 10]].

Test for Alkaloids

To 2 ml of plant extract, 1.5 ml of 1% HCl was added. After heating the solution in water bath, 6 drops of Mayors reagents/ Wagner’s reagent/ Dragendroff reagent was added. Formation of Orange precipitate indicates the presence of alkaloids [9, 11, 12].

Test for Cardiac Glycosides (Keller-Killani Test)

To 5 ml of the plant extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. Then it was underplayed with 1 ml concentrated sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardio glycosides. A violet ring may appear below the ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer [9, 10].

Test for Phenols

To 2 ml of the plant extract, 1 ml of 1% ferric chloride solution was added. Blue or green colour indicates the presence of phenols [9, 10, 13].

Test for Flavonoids

A portion of the plant extract was separately heated with 10ml of ethyl acetate in a water bath for 3min. The mixture was filtered and 4ml of each filtrate were shaken with 1ml of dilute ammonia solution. A yellow colour observation indicates the presence of flavonoids [9,14].

Phytochemical Analysis

Determination of tannin

Tannin content was determined according to the method described by Makkar and Goodchild [15]. About 0.2 g of the sample was weighed into a sample bottle, 10 ml of 70% aqueous acetone was added and properly covered. The bottle was put in an ice bath shaker and shaken for 2 h at 30 ºC. The solution was centrifuged and the supernatant stored in ice. Approximately 0.2 ml was pipette into a test tube and 0.8 ml of distilled water was added. Standard tannic acid solution was prepared from 0.5 mg/ml of the stock and the solution made up to 1 ml with distilled water. 0.5 ml of Folin Ciocalteu's reagent was added to the sample and standard followed by 2.5 ml of 20% Na_2CO_3. The solution was vortexed and incubated for 40 min at room temperature, its absorbance was read at 725 nm. The concentration of tannin in the sample was calculated from the standard tannic acid curve.

Determination of alkaloid

Alkaloid content was determined by the method of Harbone [14]. Five (5 g) of the sample was weighed into a 250 ml beaker and 50 ml of 10% acetic acid in ethanol was added, shaken and

allowed to stand for 4 h. This was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle; the precipitate was collected, washed with 1% ammonium hydroxide and filtered. The residue was dried at 60 ºC for 30 min and weighed. The weight of alkaloid was determined by weight difference and expressed as a percentage of the sample weight analysed.

$$\% \text{ Alkaloids} = \frac{W2 - w1}{w} x100$$

Where:

w= weight of sample

$w2$= weight of empty filter paper

w_1= weight of paper + precipitate

Saponin Content

Exactly 5 g of the sample was added into 50 mL of 20 % ethanol. Heat was applied to the suspension with the use of hot water bath for 1 hour with constant stirring at 55 ºC. The sample suspension was filtered and 50 mL of 20 % ethanol will be used to re-extract the residue. The collective sample extract was concentrated to 20 mL over hot water bath at about 90 ºC. The concentrated solution acquired was shaken energetically with 10 mL of diethyl ether using a separating funnel. The aqueous layer was poured into a beaker while the ether layer was discarded. 20 mL of butanol was added to the filtrate and washed thrice with 10 mL of 5% w/v sodium chloride. The mixture was heated to evaporation on hot water bath and oven dried to a constant weight. The percentage saponin content of the sample will be calculated using the following equation as described by Okwu and Emenike [16].

% Saponins = Weight of final filtrate / weight of sample × 100

Total Phenolic Content (TPC)

Total phenolic content of the extracts was determined using method of Sigleton et al. [17]. Exactly 0.5 mL of each extract (1 mg/mL) was mixed with 2.5 mL Folin-Ciocalteu reagent (1:10 v/v) and 2 mL of 7.5 % w/v of sodium carbonate. The mixture was vortexed for 15 seconds and incubated for 30 minutes at 40 ^{0}C for color development. With Gallic acid as the standard, a

calibration curve will be obtained. Absorbance was measured at 765 nm using Selecta UV/Visible spectrophotometer. The total phenolics content was expressed as mg/g Gallic acid equivalent (GAE) using the equation obtained from the calibration curve.

Total Flavonoid Content

The total flavonoid content of the samples was estimated using the method of Ordonez et al. [18]. Exactly 0.5 mL of 2 % Aluminum chloride ($AlCl_3$) prepared in ethanol was mixed with 0.5 mL of each sample (1mg/mL). The resulting mixture was incubated for 60 min at room temperature for color development (yellow) to confirm the presence of flavonoid. With quercetin as standard, calibration curve was obtained. Absorbance was measured at 510 nm using UV/visible spectrophotometer. The total flavonoids content was expressed as mg/g Quercetin equivalent (QE) using the equation obtained from the calibration curve.

Proximate Analysis

The samples were analyzed for moisture, ash, crude fibre, crude protein (N x 6.25), crude fat and carbohydrate according to the procedures described by AOAC [19].

RESULTS AND DISCUSSION

Table 1 shows the photochemical screening of *Acalypha wilkesiana* leaves dried at 50 ºC and 80 ºC. *Acalypha wilkesiana* leaves' samples revealed the presence of terpenoids, steroids, tannins, alkaloids, cardiac glycosides, phenol and flavonoid. Table 2 showed the phytochemical contents of *Acalypha wilkesiana* leaves dried at different temperatures. Table 3 shows the proximate composition of oven dried sample of *Acalypha wilkesiana* leaves at different temperatures.

Each phytochemical has been reported to display potency towards some biological action; for example, compounds such as terpenoids, tannins, steroids, and saponins show the potential towards antimicrobial (both bacterial and fungus) activity [20] Alkaloids are important in antimicrobial, analgesic, and other antispasmodic actions [21-23]. Flavonoids play a role in antioxidant potential [21]; and inflammatory potency was found with steroids [22]. Steroids make the lipid-bilayer membrane rupture and release liposome [24] while terpenoids are involved in the weakening of cell wall and tissue of the microorganisms [25].

Table 1: Phytochemical Screening of *Acalypha wilkesiana* leaves dried at 50 ºC and 80 ºC

Phytochemicals	Test	50 ºC	80 ºC
Terpenoids	Chloroform and H_2SO_4	++	++
Steroids	Acetic anhydride and H_2SO_4	++	++
Tannins	Ferric chloride test	++	++
Alkaloids	Wagner's test	++	
Cardiac glycosides	Glacial acetic acid and Ferric chloride solution	+	+
Phenols	Ferric chloride test	++	++
Flavonoids	With ammonia solution	++	+

A= sample of *Acalypha wilkesiana* leaves dried at 50 ºC, B=sample of *Acalypha wilkesiana* leaves dried at 80 ºC, + = present, - = absent, ++ = abundantly present

Table 2: Phytochemical Analysis of the *Acalypha wilkesiana* leaves dried at 50 ºC and 80 ºC

Parameter	50 ºC	80 ºC
Phenolic (mg GAE/g)	58.77±0.23	31.33±0.01
Flavonoid (mg QE/g)	23.39±0.29	18.06±0.05
Saponin (%)	12.51±0.01	12.04±0.05
Tannin (mg/g)	45.59±0.03	44.53±0.03
Alkaloids (%)	5.68±0.21	5.51±0.17

All values are means of triplicate determination ± standard deviation (SD).

A= sample of *Acalypha wilkesiana* leaves dried at 50 ºC, B= sample of *Acalypha wilkesiana* leaves dried at 80 ºC

Table 3: Proximate composition of *Acalypha wilkesiana* leaves dried at 50 ºC and 80ºC

Parameter	A	B
Moisture (%)	12.00±0.10	11.00±0.02
Ash (%)	9.75±0.02	8.00±0.00
Fibre (%)	0.50±0.00	0.50±0.00
Fat% (%)	3.50±0.20	5.50±0.00
Protein (%)	11.63±0.02	12.10±0.00
CHO (%)	62.62±0.00	62.92±0.02
Energy (Kcal)	328.50±0.02	349.50±0.00

All values are means of triplicate determination ± standard deviation (SD).

A= sample of *Acalypha wilkesiana* leaves dried at 50 C, B= sample of *Acalypha wilkesiana* leaves dried at 80 ºC

The quantitative phytochemical composition of the *Acalypha wilkisiena* leaves at 80 ºC and 50 ºC were 31.33 mg GAE/g and 58.77 mg GAE/g, 18.06 mg QE/g and 23.39 mg QE/g, 12.04% and 12.51%, 44.58mg/g and 45.59 mg/g, 5.51% and 5.68% for phenolic, flavonoid, saponin, tannin, and alkaloids respectively. Generally, the samples dried at 50 ºC were observed in high quantity than the sample dried at 80 ºC. Phenolic have found to inhibit autoxidation of unsaturated lipids, thus preventing the formation of low-density lipoprotein (LDL), which is considered to induce cardiovascular disease [26]. Flavonoids content have been implicated in the prevention of allergies and ulcers [27]. Saponin has properties of precipitating and coagulating red blood cells, cholesterol binding properties and formation of foams in aqueous solutions [28]. Plant alkaloids and the synthetic derivatives are used as a basic medicinal agent due to their analgesic, antispasmodic and antibacterial properties [29].

The results of the moisture content were 12.00±0.10 and 11.00±0.02 for 50 ºC and 80 ºC. Moisture content of oven dried sample at 80 ºC was significantly lower than that of 50 ºC which showed that increase in drying temperature results in decreased moisture contents. Moisture content values of ≤12% have been reported to be adequate for shelf life stability (up to one year) for dry vegetables [30].

The results of the ash content were 9.75% and 8.00 % for 50 ºC and 80 ºC. The ash content of the sample dried at 50 ºC was higher than the ash content of sample dried at 80 ºC. This is in agreement with Akah et al [31] who also observed reduction in in ash content of *Ocimum basilicum* l. as drying temperature increases. The fiber content of the sample had the same value of 0.5% for sample A and B. This showed that increased in temperature had no effect on the fiber content of the sample. The fat, protein and carbohydrate contents for sample A-B increased from 3.50 - 5.50%, 11.63-12.10% and 62.62 -62.92%. According to Schneeman [32], crude fibre contributes to the health of the gastrointestinal system and metabolic system in man. The considerable percentage of fat, protein and carbohydrate in this study implies that *Acalypha wilkesiana* is a nutrient dense leaf which can be used as an energy source by using it as a supplement in food. This may help to meet global demand for food security.

CONCLUSION

From the study, it can be concluded that temperature has effect on the phytochemical and proximate analysis of the *Acalypha wilkesiana* leaves. Sample A dried at 50 ºC have more

phytochemical content than sample B dried at 80 ºC. The findings revealed that bioactive compounds such as phenolic, flavonoid, saponin, tannin and alkaloids which are more bioavailable at lower temperature will demonstrate great pharmacological activities. The ash content of the sample A was higher than that of sample B. Sample B has higher percentage of fat, protein and carbohydrate. The results of the analysis depict that *Acalypha wilkesiana* leaves offer great medicinal and nutritional advantage. Further studies need to be carried out on the in-vivo pharmacological activities of the extract of *Acalypha wilkesiana* leaves so as to expound more on the medicinal potency of the leaves.

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