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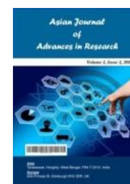

COMPARATIVE STUDY ON THE ECOLOGICAL EFFECT OF DIFFERENT DRYING METHODS ON THE PHYTOCHEMICAL CONSTITUENTS OF THE LEAVES OF TRIDAX PROCUMBENS LINN

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AUTHORS' CONTRIBUTIONS

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ABSTRACT

The role of plants in medicine cannot be over-emphasized; nearly all known conventional drugs have their composition to originate from plants. This medicinal properties possessed by plants can be attributed to some chemicals which are called phytochemicals produced in some parts of the plants. The ease in access to plants which is all around us, made individuals and households to become Doctors of their own. In that the hear-say knowledge of plants has led to people choosing plants for treatment of sicknesses over conventional drugs. Locally, there are various ways individuals prepare their herbal medicine, and they include; boiling, drying under the sun and air drying etc. This study was aimed to know the extent to which some of these methods of herbal medicine preparation (sun drying and air drying) affect the phytochemical composition of some plant parts. The leaves of Tridax procumbens were used for this study, and it was evaluated for total saponin, total flavonoids and other secondary metabolites using standard procedures. The study of two different samples from the aforementioned two different methods of herbal medicine preparation reveals the presence of saponin, tannin, alkaloid, flavonoid, steroid. The air dried sample gave higher composition of saponin (1.85±0.03), flavonoids (1.34±0.03), tannin (2.51±0.02), alkaloids (1.49±0.02) and steroid (0.26±0.02), there is significant difference between the phytochemicals seen in the two samples. Thus, this reveals that the air drying method will have little effect on the phytochemical constituent of the leaves, and can be preferred over sun drying in preparation of herbal medicine.

Keywords: Phytochemical; composition; plants; extraction; drying; herbal.

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1. INTRODUCTION

"There is a strong connection between man and nature, ever since his first origin. Man has discovered the benefit of plant kingdom, which he used to feed himself, to heal and to survive" (Santic *et al.,* 2017). "Primitive people have depended on nature for food, shelter, clothing and medicine to cure ailments. These made humans distinguish useful herbs with beneficial effects from those that were inactive or toxic" [1]. "The practice of herbal medicine is the oldest form of healthcare which has been used for decades in developing and developed countries. Plant based drugs awareness advanced gradually and has been passed on, therefore setting a foundation for many traditional medicine systems around the globe" [1]. "The pharmacological treatment of disease began long ago with the use of herbs" [2]. "Today herbal medicine is still the primary healthcare system for about 80% of the world's population, especially in the developing countries" [1[, (Okigbo et al., 2009).

"According to literature approximately 50,000 plant species have medicinal properties" [3]. "Thus, the basis of modern medicinal drugs such as aspirin, morphine, digitoxin and quinine were synthesized through scientific validation of herbal medicine" [4,5]. "In the middle Ages, there were written many herbal manuals that described the use and procedures in healing with plants were known by the oldest civilizations and they were used by the people for thousands of years. Moreover, today's science has confirmed their effectiveness in the treatment of different diseases" [6].

1.1 Herbal Medicine?

"The World Health Organization (WHO) defines herbal medicine as a practice which includes herbs, herbal materials, herbal preparations and finished herbal, that contain as active ingredients parts of plants, or other plant materials, or combinations. These herbs are derived from plant parts such as leaves, stems, flowers, roots, and seeds" [7].

1.2 Some Plants with Medicinal Value

The following are few plants out of the vast number of plants with medicinal value.

1.3 Garlic

"Traditionally, garlic (*Allium sativum*) has been used to treat colds, chronic bronchitis, coughs, respiratory catarrh, bronchitic asthma and influenza. Additionally, it is used mainly to manage hypertension and hypercholesterolemia." [8]. "It has been noticed that garlic lowered the blood pressure and level of cholesterol and also possesses strong antimicrobial activity" [9]. "It contains alliin, which upon chopping or crushing is activated by alliinase in the absence of acid or heat" [10,11]. "Allicin produces both hydrophilic (cysteine) and lipophilic (sulfides, ajoene) sulfur compounds which are accountable for pharmacologic effects. Garlic is administered via oil-filled capsules, condensed dried powder, and enteric-coated tablets and capsules; it is also added in aqueous alcohol" [11].

1.4 *Echinacea purpurea*

"The species *Echinacea purpurea* from this genus is a well-known medicinal plant used in treating snake bites, toothache, skin disorders, bowel pain, chronic arthritis, seizure and cancer, traditionally" [12]. "*E. purpurea* possesses secondary metabolites including caffeic acid derivatives, alkamides, glycoproteins and polysaccharides alleged to be biologically and pharmacologically active" [8, 12].

1.4.1 Konjac (*Amorphophallus konjac*)

"It is known to be a significant dietary source of glucomannan" [13], "which is used in treating obesity, Also used constipation and reducing cholesterol" (Chen *et al*., 2003).

1.4.2 Paw paw (*Carica papaya*).

"Used for treating wounds and stomach troubles" (Gurung and Skalko-Basnet, 2009).

1.4.3 Arnica (*Arnica montana*).

Used as an anti-inflammatory (Dal Sasso *et* al., 2006) and for osteoarthritis (Widrig *et al*., 2007).

1.5 Methods of Herbal Medicine Preparation

1.5.1 Maceration

Maceration is an extraction procedure in which coarsely powdered drug material, either leaves or stem bark or root bark, is placed inside a container; the menstruum is poured on top until completely covered the drug material. The container is then closed and kept for at least three days. The content is stirred periodically, and if placed inside bottle it should be shaken time to time to ensure complete extraction. "At the end of extraction, the micelle is separated from marc by filtration or decantation. Subsequently, the micelle is then separated from the menstruum by evaporation in an oven or on top of water bath" [14,15]. This method is convenient and very suitable for thermolabile plant material.

1.5.2 Infusion

"This is an extraction process such as maceration. The drug material is grinded into fine powder, and then placed inside a clean container. The extraction solvent hot or cold is then poured on top of the drug material, soaked, and kept for a short period of time" [14,16], Pandey & Tripathi, 2015; [17] "This method is suitable for extraction bioactive constituents that are readily soluble. In addition, it is an appropriate method for preparation of fresh extract before use. The solvent to sample ratio is usually 4:1 or 16:1 depending on the intended use" [14,16,18,17].

1.5.3 Decoction

"This is a process that involves continuous hot extraction using specified volume of water as a solvent. A dried, grinded, and powdered plant material is placed into a clean container. Water is then poured and stirred. Heat is then applied throughout the process to hasten the extraction" [14,16,18,17]. "The process is lasted for a short duration usually about 15 min. The ratio of solvent to crude drug is usually 4:1 or 16:1. It is used for extraction of water soluble and heat stable plant material" [14,16,18,17].

1.5.4 Digestion

"This is an extraction method that involves the use of moderate heat during extraction process. The solvent of extraction is poured into a clean container followed by powdered drug material. The mixture is placed over water bath or in an oven at a temperature about 50°C" [14,18,17]. "Heat was applied throughout the extraction process to decrease the viscosity of extraction solvent and enhance the removal of secondary metabolites. This method is suitable for plant materials that are readily soluble" [14,18,17].

1.5.5 Percolation

"The apparatus used in this process is called percolator. It is a narrow-cone-shaped glass vessel with opening at both ends. A dried, grinded, and finely powdered plant material is moistened with the solvent of extraction in a clean container. More quantity of solvent is added, and the mixture is kept for a period of 4 h. Subsequently, the content is then transferred into percolator with the lower end closed and allow to stand for a period of 24 h" [16,18,17]. "The solvent of extraction is then poured from the top until the drug material is completely saturated. The lower part of the percolator is then opened, and the liquid allowed to drip slowly. Some quantity of solvent was added continuously, and the extraction taken place by gravitational force, pushing the solvent through the drug material downward" [16,18,17]. "The addition of solvent stopped when the volume of solvent added reached 75% of the intended quantity of the entire preparations. The extract is separated by filtration followed by decantation. The marc is then expressed and final amount of solvent added to get required volume" [16,18,17].

Risks associated with the use of herbal medicines.

Some risks associated with the use of herbal medicines are;

1.5.6 Nephrotoxicity (Kidney damage).

"Herbal medicine is believed to be capable of causing nephrotoxicity due to may be addition of toxins during careless preparation, addition of adulterants, heavy metals, and some pharmaceutical products intentionally to reduce cost or increase efficacy" [19]. **"**Herbs such as *Tripterygium wilfordii* Hook (thunder god vine) contain diterpenoid epoxide, which induces apoptosis causing kidney damage. *Averrhoa carambola* L. (star fruit) contains oxalate in high quantity, which can cause acute nephropathy. *Guaiacum officinale* L. (rough bark) and *Arctostaphylos uva-ursi* (cranberry) increase stone formation. *Aristolochia fangchi* causes well known aristolochic acid nephropathy. *Callilepis laureola* DC (Impila) inhibits mitochondrial ATP synthesis. *Uncaria tomentosa* wild DC (Peruvian's Cat Claw) causes acute allergic interstitial nephritis. Studies are being conducted on *Salix alba* L. (willow bark) analgesic nephropathy induction. *Ephedra sinica* Stapf (Chinese ephedra) affects reninangiotensin-aldosterone system. *Glycyrrhiza glabra* L. (Licorice) and *Harpagophytum procumbens* DC (devil's claw) inhibit renal transport processes" [20].

1.5.7 Hepatotoxicity (Liver damage or poison)

"The severity of this toxicity is widely variable between mild hepatitis to acute hepatic failure. The scoring system for allopathic medicines can be assessed but is not suitable for herbal medicines and needs validation. Many Ayurvedic and Chinese herbal medicines are reported to cause hepatotoxicity" [21]. "Major hepatotoxic herbs are *Cimicifuga racemosa* (black cohosh), *Larrea tridentata* (chaparral), *Teucrium chamaedrys* (germander), *Scutellaria lateriflora* (American skullcap), and *Scutellaria baicalensis* (Chinese skullcap), etc" [22].

1.5.8 Cardiotoxicity (Heart damage)

"Cardiotoxic agents include chemotherapeutic drugs of anthracycline class, alkylating agents such as

cyclophosphamide, cisplatin, chlormethine, mitomycin, etc. Some other agents such as paclitaxel, etoposide, fluorouracil, asparaginase, tretinoin, pentostatin may cause cardiotoxicity" [21]. "This may be increased due to cumulative dose, rate, and schedule of administration, history of preexisting cardiovascular problems, and disturbed balance of cardiac electrolytes" [23]. "Herbal medicines having direct effect on heart include medicine prepared from plants such as *Digitalis purpurea* (digitalis), *Catharanthus roseus* (vinca), *Aconitum napellus* (monk's hood), *Atropa belladonna* (deadly nightshade), *Ephedra distachya* (sea grape), *Mandragora officinarum* (mandrake), *Glycyrrhiza glabra* (licorice), etc" [24].

1.6 Aim of Study

The aim of the study is to determine the effect of sun drying and the air drying methods on the phytochemicals present in the leaves of *Tridax procumbens* in herbal medicine preparation by individuals or household.

2. MATERIALS AND METHODS

2.1 Experimental Site

This was carried out at the department of Botany laboratory, Nnamdi Azikiwe University, Awka, Anambra State.

2.2 Instruments/Equipment Used

Petri dish, Weighing balance, Blender, Knife, Water bath, volumetric flask

2.3 Chemicals/Reagents Used

Ether, methanol, distilled water, ammonia, suphuric acid, meyer`s reagent, ferric chloride, sodium chloride, acetic anhydride, folin-dennis reagent, sodium carbonate, ethanol, tanuric acid, N-hexane, chlorofoam.

2.4 Source of Plant Materials

2.4.1 Sample collection

Leaves of *Tridax procumbens* were collected from the surrounding of Nnamdi Azikiwe University, Awka South L.G.A, Anambra State, Nigeria. The name of the plant was authenticated.

2.4.2 Sample preparation

The plant was examined to be free from disease. Only healthy leaves were used. Non-essential materials attached to the plant part were removed, and then divided into two parts. The first part was sun dried for two days to serve as a sample for the sun drying herbal medicine preparation. While the other part was air dried under room temperature for two days.

2.5 Methodology

2.5.1 Phytochemical screening

The phytochemical screening was carried out according to the method of [18].

2.5.2 Test for Saponin

2g of the powdered sample was boiled in 20ml of distilled water in a water bath for five minutes. The filtrate was mixed with 5ml of distilled water and shook vigorously and formation of foam was observed.

2.5.3 Test for Flavonoids

5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of the plant extract followed by the addition of concentrated H2SO4. A Yellow colouration which indicates the presence of flavonoids was observed.

2.5.4 Test for Phenols

2ml of distilled water was added to two millilitres of plant extract followed by the addition of 10% FeCl2. Bluish black colour indicates the presence of phenol.

2.5.5 Test for Alkaloids

0.5g of the sample was defatted with 5% ethyl ether for fifteen minutes. The defatted samples were boiled for 20minutes with 5ml of HCl in a water bath. The resulting mixture was centrifuged for ten minutes at 3000rpm. The filtrate was then treated with few drops of Meyer's reagent and turbidity observed.

2.5.6 Test for Steroids

2ml of acetyl anhydride was added to 0.5g of the ethanolic extract with two millilitres of H2SO4. Blue-green coloration was observed which indicated the presence of steroid.

2.5.7 Test for Tannins

0.5g of the powdered sample was boiled in 20ml of distilled water and filtered. A few drop of 0.1% of ferric chloride was added and brownish-green or blue-black coloration was observed.

2.5.8 Test for Glycoside

5ml of extract was treated with 2ml of glacial acetic acid; Ferric chloride acid was added in drops than addition of 1ml of sulphuric acid. Formation of brown ring was observed.

Quantitative determination of the phytochemical constituent of the plant parts studied.

2.5.9 Alkaloid determination

The determination of the concentration of alkaloid in the sun dried and air dried leaves of *Tridax procumbens* was carried out using the alkaline precipitation gravimetric method described by Harbone (1973). Five grams of the powdered samples was soaked in 20ml of 10% ethanolic acetic acid. The mixture was stood for hours at room temperature. Therefore, the mixture was filtered through whatman filter paper, (NON2). The filtration was concentrated by evaporation over a steam bath to one quarter of its original volume. To precipitate the alkaloid, concentrated ammonia solution was added in drops to the extract until it was excess. The resulting alkaloid precipitate was washed with 9% ammonia solution and dried in the oven at 60°C for thirty minutes, cooled in a desiccator and reweighed. The process was repeated two more times and the average was taken. The weight of the alkaloid was determined by the differences and expressed as a percentage of weight of sample analyzed as shown:

$$\% \text{ Alkaloid} = \frac{W2-W1}{WEIGHT\ OF\ SAMPLE} \times \frac{100}{1}$$

Where:

W1= weight of filter paper
W2= weight of filter paper + alkaloid precipitate.

2.5.10 Saponin determination

20g of the sample was extracted with 100ml of 20% ethanol in a conical flask. The extract was heated over a hot water bath for four hours with continuous stirring at about 55°C. The mixture was then filtered and residue re-extracted with another 200ml of 20% ethyl alcohol. The combined extracts were reduced to 40ml and then boiled over a water bath at about 90°C, the concentrate was then transferred into a two hundred and 50ml conical flask and 20ml of diethyl ether was added to the extract and vigorously shaken. The aqueous layer was recovered while the diethyl ether was discarded and purification process i.e. washing twice with 10ml of 5% sodium chloride was carried out. The remaining solution was then heated in a water bath and allowed to evaporate, the sample was dried in the oven to a constant weight and values expressed as mg/g of extract.

$$\% \text{ Saponin} = \frac{W2-W1}{WEIGHT\ OF\ SAMPLE} \times \frac{100}{1}$$

Where

W2-W1 = Weight of Saponins
W1= weight of conical flask
W2= Weight of conical flask+ Saponins

2.5.11 Flavonoids determination

The Flavonoids content was determined by the gravimetric method as was described by Harbone (1973). 5g of the powdered sample was placed into a conical flask and 50ml of water and 2ml of HCl solution was added. The solution was allowed to cool before it was filtered through whatman filter paper. The filtrate was treated with ethyl acetate starting with drops of it, until in excess. The precipitated flavonoids were recovered using a weighed filter paper, and then placed in an oven to dry at 80°C. It was cooled in a desiccator and reweighed. The difference in weight gave the weight of the flavonoids which was expressed in percentage of the sample weight analyzed. Given by the formula.

$$\% \text{ Flavonoids} = \frac{W2-W1}{WEIGHT\ OF\ SAMPLE} \times \frac{100}{1}$$

Where:

W= weight of sample
W1= weight of empty filter paper
W2= weight of paper + flavonoids extract.

2.5.12 Steroids determination

This was carried out using the Salkowki test as described by Harbone (1998), 5g of the sample was extracted with 20ml of distilled water and agitated in a vibrator at relatively low speed for three hours, after which it was allowed to stand overnight. The mixture was then put into a beaker with the aid of whatman grade filter paper. The filtrate was eludated with 10ml of NH2OH and 2ml of the filtrate was mixed with 2ml of chloroform. This was followed by the addition of 3ml of acetic anhydride and 2ml of concentrated sulphuric acid in drop. The combined extracts were diluted with 100ml of distilled water and were dispensed into 250ml conical flask containing 10g of the sample. Then the absorbance was read in UV/ visible spectrophotometer at the wavelength of 420nm. The steroid content was calculated as show below:

$$\% \text{ steroids} = \frac{100}{W} \times \frac{AU}{AS} \times \frac{C}{100} \times \frac{VF}{VA} \times D$$

Where:

W= weight of sample analyzed
AU= absorbance of test sample
AS= Absorbance of standard solution
C= Concentration of standard in mg/ml
VF= total filtrate volume
VA= Volume of filtrate analyzed
D= Dilution factor.

2.5.13 Tannin determination

The tannin content of the plants was determined using Folìn-Dennis spectrophotometric method described by (Onwuka, 2018). 2g of the powdered sample was mixed with 50ml of distilled for 50minutes in a shaker. The mixture was filtered and the filtrate used for the experiment. 5ml of the filtrate was measured into 50ml volumetric flask and diluted with 3ml of distilled water. Similarly, 5ml of standard tanuric acid solution and 5ml of distilled water were added separately. 1ml of Folìn-Dennis reagent were added to each of the flask, followed by 2.5ml of saturated sodium carbonate solution. The content of each flask was made up to mark and incubated for 90minutes at room temperature. The abundance of the developed colours was measured at 760nm wavelength with reagent blank at zero. The process was repeated two more times to get an average. The tannin content was calculated as follows:

$$\% \text{ Tannin} = = \frac{100}{W} \times \frac{AU}{AS} \times \frac{C}{100} \times \frac{VF}{VA} \times D$$

Where:

W= weight of sample analyzed
AU= absorbance of test sample
AS= Absorbance of standard solution
C= Concentration of standard in mg/ml
VF= total filtrate volume
VA= Volume of filtrate analyzed
D= Dilution factor

2.6 Statistical Analysis

The data generated was subjected to analysis of variance (ANOVA) to determine the level of significance. Significant difference (LSD) technique as described by Gomez [25] and Gomez (1980).

3. RESULTS

3.1 Qualitative Phytochemical Composition of Sun Dried and Air Dried Leaves of *Tridax procumbens*

Results of the qualitative phytochemical composition of sun dried and air dried leaves of *Tridax procumbens* are presented in Table 1. The result revealed the presences of saponin, steroid, alkaloids, tannins and flavonoids.

Table 1. Qualitative phytochemical composition of sun dried and air dried leaves of *Tridax procumbens*

Phytochemicals	*Tridax procumbens* (Linn)	
	Sun dried leaves	Air dried leaves
Saponin	+	+
Flavonoid	+	+
Alkaloid	+	+
Steriod	+	+
Tannin	+	+
Phenol	-	-
Glycoside	-	-

***Key:** +: Present, -: Absent*

3.2 Quantitative Phytochemical Composition of Sun Dried and Air Dried *Tridax procumbens*

Table 2. Quantitative phytochemical composition of sun dried and air dried leaves of *Tridax procumbens*.

Plant samples	Saponins (mg/100g)	Flavonoids (mg/100g)	Tannin (mg/100g)	Alkaloids (mg/100g)	Steroids (mg/100g)
Air dried Leaves	1.85±0.03	1.34±0.03	2.51±0.02	1.49±0.02	0.26±0.02
Sun dried leaves	1.71±0.02	1.23±0.03	2.43±0.04	1.42±0.02	0.16±0.03
LSD	0.125	0.307	0.0306	0.062	0.053
P-Value	0.000	0.000	0.00	0.000	0.000

Results are represented in Mean ± Standard deviation (P-value ≤0.05 shows there is significant difference).

The quantitative phytochemical compositions of the air dried and sun dried leaves of *Tridax procumbens* are shown in Table 2. The Table revealed that the air dried leaves of *Tridax* contains (1.85±0.03 mg/100g) of total saponins, (1.34±0.03 mg/100g) of total flavonoids, (2.51±0.02 mg/100g) of total tannin, (1.49±0.02 mg/100g) of total alkaloid, (0.26±0.02 mg/100g) of total steroid. The sun dried leaves then contains (1.71±0.02 mg/100g) of total saponin, (1.23±0.03 mg/100g) of total flavonoids, (2.43±0.04 mg/100g) of total tannin, (1.42±0.02 mg/100g) of alkaloids, (0.16±0.03 mg/100g) of total steroid. There is significant difference between the phytochemicals present in the two samples.

4. DISCUSSION AND CONCLUSION

4.1 Discussion

"The phytochemical screening results in Table 1 showed that saponins, flavonoids, alkaloids, tannins and steroids are present in both the sun dried and the air dried leaves of *Tridax procumbens,* while phenols and glycosides are absent in both the air dried and the sun dried leaves of *Tridax procumbens".*

"The presence of saponins signifies that the plant could be an important source for tumor effect, flavonoids have antioxidant and steroids have anti-inflammatory properties as recorded by Aderibigbe et al., [26]. According to [27], alkaloids in medicine are used in the treatment of cancer, parasitic diseases, pathogenic bacteria and neuronal disorders. As recorded by Nnam *et al*., (2012), saponins are glucosides with foaming characteristics. Saponins consist of polycyclic aglycones attached to one or more sugar side chains. It has a bitter taste; it has antifungal properties," [26].

Quantitatively, tannin was the highest occurring phytochemical in the sun dried and air dried leaves at (2.43±0.04 and 2.51±0.02) respectively, while steroid was the least occurring phytochemical in the sun dried and air dried leaves at (0.16±0.03 and 0.26±0.02) respectively. WHO guidelines on 'Good herbal processing practices for herbal medicines' states that any method used to concentrate the extracts must avoid excessive heat because the active ingredients may be heat labile [28]. In present study, the results clearly show that sun drying method has an effect on the phytochemical constituents of all the parts of the plant studied. The longer the exposure of these plant parts to the heat of the sun, the more the damage to these phytochemical constituents.

4.2 Conclusion

Sun drying and air drying which are also methods of household use in the preparation of herbal medicine in Nigeria are both acceptable for the preparation of herbal medicine. Although the heat of the sun can affect the phytochemical composition of a plant part, depending on the length of time of exposure to heat of the sun. Hence, the herbal processing practice involves minimization of loss and/or damage to the chemical constituents of interest ensuring the effectiveness of the preparation. Therefore, lesser heat is required during the process of herbal medicine preparation, especially during concentration process by evaporation, using any type of heat.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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