



Phytochemical, Proximate and Elemental Composition of *Tithonia diversifolia* (Hemsley) A. Gray leaves

Omolola, T.O

Forestry Research Institute of Nigeria



* Corresponding author email: oyebee710@gmail.com

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ABSTRACT

In recent times, medicinal plants are being greatly used ethnomedicinally for the management and treatment of diseases and health conditions. One of such medicinal plants is *Tithonia diversifolia*. This study aimed at determining the phytochemical, elemental and proximate content of the powdered plant part to help infer what is responsible for the reported therapeutic characteristics of the plant as well as determine the quality, purity and safety profile of the plant. Leaves of *T. diversifolia* were subjected to phytochemical, proximate and mineral screening using standard laboratory procedures. Qualitative phytochemical observation revealed the presence of Tannins, saponins, flavonoids and terpenoids. Crude Protein content was the highest with 27.69% followed by Crude Fibre (14.89%), Ash (13.86%), Moisture Content (10.92%) and the least value 1.14% for Fat. Magnesium (Mg) content was highest with 6712.50mg/kg which is 22, 35, 122 & 144 times higher than the content of Iron (Fe) (291.5mg/kg), Manganese (Mn) (191.0mg/kg), Zinc (Zn) (54.9mg/kg) and Copper (Cu) (15.1mg/kg). However, elements (heavy metals) Lead (Pb), Cadmium (Cd), Chromium (Cr) and Cobalt (Co) were not detected. Results obtained in the study is indicative of a potential drug substitute which can be used to help manage and treat diseases due to the phytochemicals, nutrients, and minerals present in them. Also, the safety indices of this plant part have been established, thus, information obtained from the study can serve as a reference standard for the plant monograph.

Keywords: *Tithonia diversifolia*, Phytochemicals, Minerals, Heavy metals, Proximate

1 Introduction

Tithonia diversifolia (Hemsley) A. Gray is a representative of the sunflower family (Asteraceae) and it is popularly called Tree Marigold, Mexican Sunflower, Japanese Sunflower [1]. It is a perennial or annual shrub that is native to North and Central America, but is naturalised in Africa, Australia, and Asia, where it is an aggressively invasive species [2]. It is a prolific weed, which grows aggressively abandoned farmland, along road paths all over Nigeria. Due to the invasive nature of this plant in Nigeria, farmers have abandoned their lands as a result of the challenges experienced in ensuring the Mexican sunflower does not take over their farms [2],[3]. Despite its invasive nature, the plant has been used as an organic fertiliser to increase

the yield of vegetable crops and maize in Nigeria and Kenya [2]. It is a woody herb/succulent shrub usually 1.2-3m tall. The leaves are simple, alternately arranged, lobed with acute or acuminate apex, crenate margins. 5-17 X 3.5-12cm in size, densely pubescent beneath with palmate venation. Flower heads are solitary on a peduncle 6-13cm in length; florets yellow [2], [4], [5]. The plant is an important medicinal plant whose leaf is the major plant part used alone or in combination with other plants for the treatment of a variety of ailments such as stomach pain, indigestion, sore throat, liver disease and pain [1],[4]. Traditionally, all parts of the plant especially the leaves, are widely used by indigenous people for treating a wide spectrum



of ailments and diseases ranging from topical application—to treat wounds, skeleto-muscular disorders, abscesses, dermatological conditions, and stomach pains—to oral administration for diabetes, malaria, fever, hepatitis and infectious diseases [6]. In Indonesia, the decoction of the aqueous extract of the plant is used for the treatment of diarrhoea, diabetes, stomach ache and wounds [2], [7]. In Nigeria, the leaf is used for managing and treating malaria [6]. Leaf infusion in water is taken orally to cure menstrual pain [2], [8]. Oral administration of cold water leaf infusion is used to treat malaria. It is applied topically as poultice or bath to wounds, bruises, skeletomuscular disorders, abscesses, dermatological conditions, and stomach pains [1]. Reported pharmacological activities include antiplasmodial [1], Antiinflammatory, antidiabetic [9], antioxidant [10], [11], [12], antibacterial [1], [13], antidiarrheal [1], acute toxicity [1], antimalarial [1], [14], hepatoprotective [1], anticancer, antiulcer, toxicology and cytotoxicity [1]. Phytochemical detected from the leaves includes phenolics, flavonoids, saponins, alkaloids, steroids, and tannins [15].

Qualitative and quantitative determination of mineral elements present in plants is important because they play a crucial role against a variety of degenerative diseases. It is also important to note that some minerals are essential to a healthy diet (e.g. Magnesium Calcium, Phosphorus, Potassium and Sodium) while some can be toxic (e.g. Lead, Mercury, Cadmium and Aluminium). Thus, the knowledge of the elemental content of plants is needed because it may influence their active constituents' production and their pharmacological action. Active constituents of medicinal plants are metabolic products of plant cells and a number of trace elements are responsible for this metabolism [16].

Proximate evaluation provides valuable information on the nutritional content as well as accessing the quality of the sample such as moisture content, ash, fat, crude fibre, crude protein, etc. Ash is the remaining inorganic residue after water and organic matter have been removed from any sample by heating, which provides a measure of total amount of minerals

within the sample. Minerals are not destroyed by heating and they have a low volatility as compared to other food components. Ashes give us an idea of the mineral matter contained in a plant. Thus, measuring it is important, because mineral matter may be the cause of a pharmacological effect [17].

The present investigation on the leaves of *T. diversifolia* was taken up with the aim of establishing certain botanical and chemical standards which could help in Crude drug identification, check adulteration and contribute to the existing monograph information as contained in herbal pharmacopoeias.

2 Materials and Method

2.1 Plant collection and authentication

The fresh leaves of *T. diversifolia* was collected in November, 2011, within the Staff school, University of Ibadan. The plant was authenticated by Mr Chukwuma, E.C in the Forest Herbarium, Institute (FHI) of Forestry Research Institute of Nigeria (FRIN), Ibadan, where voucher specimens were deposited. The plant was assigned a voucher specimen number FHI 109598.



Figure 1: *T. diversifolia* growing in its natural habitat

2.2 Voucher Specimen Preparation

The plant part with inflorescence, fruits or seeds was collected and pressed using a plant press. They were then authenticated at FRIN by a taxonomist, further pressed to remove all the moisture, poisoned and mounted on a white cardboard paper making use of bond and paper tacks. The voucher number, province, district,

locality, habitat, description of plant, collector, date and determinavit were documented on the herbarium specimens before depositing a copy at FHI.

2.3 Phytochemical Screening

2.3.1 Test for Saponins

About 1g of the powdered sample was boiled in 5ml of distilled water in a water bath and filtered. 5ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The persistent frothing was taken as the evidence for the presence of saponins [18].

2.3.2 Test for Tannins

About 2g of the dried powdered samples was boiled in 10ml of water in a test tube and then filtered. 2-5 few drops of 0.1% ferric chloride solution was added. The occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins [18].

2.3.3 Test for Anthraquinones

2g of the plant sample was extracted with 5ml of chloroform, filtered and 5ml of 10% ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink, red, or violet colour in the ammoniacal (lower) layer indicated the presence of free anthraquinones.

Combined anthraquinones were tested for by boiling 2.5g of each extract with 2.5ml of benzene, the benzene layer separated and half its own volume of 10% NH₃ solution added. A pink, red or violet coloration in the ammonia lower phase indicated the presence of derivatives of anthraquinone [18].

2.3.4 Test for Cardiac Glycosides (Keller-Killiani Test)

1g of the plant sample was extracted with 10ml of 80% ethanol for 5 minutes on a water bath. The extract was filtered and equal volume of distilled water was added to it. A few drops of lead acetate solution were added, shaken and the precipitates were filtered off. The filtrate was extracted with three aliquots of chloroform. The chloroform extract was divided into two portions

in evaporating dish and evaporated to dryness on a steam bath.

a. Keller–Killiani’s Test: To the first portion, 3ml of ferric chloride reagent (0.3ml of 10% FeCl₃ in 50ml glacial acetic acid) was added in a clean test tube. This was then underlayered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicates the presence of a deoxy sugar characteristic of cardenolides. A violet ring appeared below the brown ring while in the acetic acid layer a greenish ring formed just above the brown ring and gradually spread throughout this layer [18].

b. Kedde test: The second portion of the residue was mixed with 1ml of a 2% solution of 3,5-dinitrobenzoic acid in ethanol and 1ml of a 5% aqueous NaOH. An immediate violet colour indicated the presence of cardenolides in the extract, the colour fading gradually through reddish – brown to brownish – yellow with the precipitation of a whitish crystalline solid. This test indicates the presence of a lactone ring in the cardenolides [18].

2.3.5 Test for Flavonoids

Shinoda’s test: 0.5g of each of the powdered sample was extracted in ethanol by boiling in a water bath for 5min, filtered and cooled. To the filtrate was added four pieces of magnesium filings followed by few drops of concentrated hydrochloric acid. A pink or red colour indicates the presence of flavonoids.

Sodium hydroxide Test: 5ml of 10% NaOH was added to an equal volume of the ethanolic extract. A yellow colouration indicates the presence of flavonoids [18].

2.3.6 Test for Alkaloids

About 1g of the plant powder was extracted with 5ml of 1% aqueous hydrochloric acid on a water bath. The pH of the filtrate was adjusted to about 6. To about 0.5ml of the filtrate few drops of Mayer’s reagent, Drageendorff’s reagent, Wagner’s reagent, 1% Picric acid solution and 10% Tannic solution were added. Turbidity or precipitation with either of these reagents was taken as evidence for the presence of alkaloids in the extract evaluated [18].

2.4 Proximate Analysis

The proximate analysis of powdered plant materials was carried out using the AOAC methods (2005) in the laboratory of the Department of Human Nutrition, Faculty of Medicine, University of Ibadan. The powdered plant sample was analysed for proximate compositions: moisture content, crude protein, crude fat, ash, crude fibre and carbohydrate [19].

2.5 Elemental analysis

Elemental analysis was done according to the method of Isaac and Korber, [20]. Briefly, 0.5g of the powdered sample was weighed into a 50ml beaker and 10ml of an acid mixture in the ratio 2:1 (HNO₃:HClO₄) was added and allowed to undergo heating on a hot panel placed in a fume cupboard for about 30 minutes until a clear solution(digest) was obtained. The digest was allowed to cool and the volume adjusted to 20ml, this was read on an atomic absorption spectrophotometer (Buck Scientific, model210/211 VLP) to determine the heavy metal composition of the sample. The elements were determined at various wavelengths viz. Zn = 213.9nm, Fe = 248.3nm, Cu = 324.8nm, Cr = 357.9nm, Co = 324.8nm, Mg = 285.2nm, Mn = 279.5nm, Ni = 341.5nm, Cd = 228.9nm, Pb = 283.3nm.

2.6 Statistical analysis

Data collected were expressed as mean \pm standard deviation of triplicate analysis.

3 Results

3.1 Phytochemical Screening

The result of phytochemical analysis is shown in Table 1.

Table 1: *Phytochemical Constituents of T. diversifolia leaves*

Constituents	<i>T.diversifolia</i>
Tannins	+
Saponin	+
Cardiac Glycosides	-
Anthraquinones	-
Alkaloids	-
Flavonoids	+
Terpenoids	+
Steroids	-

*Positive +, Negative

The phytochemical screening carried out on the leaves of *T. diversifolia* showed that it contains Tannins, Saponins, Flavonoids, Terpenoids while Alkaloids, Cardiac glycosides, Anthraquinones and steroids were found absent. The presence of these secondary metabolites is indicative of its promising array of potential pharmacological activities.

3.2 Proximate analysis

The evaluation of the proximate and physicochemical content of the leaves is represented in Table 2. The results revealed a moisture content value of 10.92%, crude protein (27.69%), Crude fibre (14.89%), Fat (1.14%), Ash (13.86%), acid insoluble ash (2.78%), water soluble ash (7.80%), alcohol soluble extractive (4.54%) and water soluble extractive (19.13%). These values helps us appreciate the proximate qualities of the understudied plant which is quite important to ascertain the quality and purity of any crude drug.

Table 2: *Proximate analysis and Physico-chemical parameters of T. diversifolia leaves*

Parameters	<i>T. diversifolia</i> (%)
Moisture content	10.92 \pm 0.05
Crude Protein	27.69 \pm 0.16
Fat	1.14 \pm 0.01
Crude Fibre	14.89 \pm 0.04
Ash	13.86 \pm 0.05
Acid insoluble ash	2.78 \pm 0.25
Water soluble ash	7.80 \pm 1.58
Alcohol soluble extractive	4.54 \pm 0.65
Water soluble extractive	19.13 \pm 0.15

*Values expressed as Mean \pm SD

3.3 Elemental analysis

The result obtained for the elemental study of *T. diversifolia* leaves is as shown in Table 3. Results revealed a very high content of 6712.50 mg/kg for Magnesium (Mg), The content of Mg is 22, 35, 122 & 144 times higher than the content of Fe (291.5mg/kg), Mn (191.0mg/kg), Zn (54.9mg/kg) and Cu (15.1mg/kg) while heavy metals Co, Cr, Cd, Pb, and Ni were not detected (ND). This result implies that *T. diversifolia* leaves have a better potential in making up for the

deficiencies of some of these elements in human body when ingested and also connotes that the plant part is highly of great potential medicinally.

Table 3: Elemental analysis of *T. diversifolia* leaves

Element	<i>T. diversifolia</i> (mg/kg)
Magnesium (Mg)	6712.50
Manganese (Mn)	191.00
Iron (Fe)	291.50
Copper (Cu)	15.10
Zinc (Zn)	54.90
Cobalt (Co)	ND
Chromium (Cr)	ND
Cadmium (Cd)	ND
Lead (Pb)	ND
Nickel	ND

*ND – NOT DETECTED

4 Discussion

Herbal medicines have received greater attention as alternatives to drug therapy, thus, leading to increase in their demand. The exclusive use of herbal drugs, prepared and dispensed by unscientifically trained herbalists, for treatment of diseases is still very common in rural Nigerian communities. Experimental screening method is, therefore, important in order to ascertain the safety and efficacy of herbal products as well as to establish the active components of these herbal remedies [21].

From the result of the phytochemical screening as represented in Table 1. The finding is in accordance with the report of Taziebou *et al* [22]. In contrast, Ezeonwumelu *et al* [23] and Olayinka *et al* [24] reported the presence of Alkaloids.

Proximate analysis results as shown in Table 2 revealed that the ash value of *T. diversifolia* leaves is 13.86%, this value is similar to the reported value of 14% [25] but higher than 8.10% as reported by Olayinka *et al* [24] which falls in the acceptable range of 22% for any standard drug [26]. Also, the value obtained was higher than the values of 6.55% and 11% obtained for *T. diversifolia* stem and leaves respectively [27]. Higher ash denotes higher mineral content, thus, we can say the plant sample understudied has a higher inorganic content than the samples used for the reported ones. Total ash value is a diagnostic index for purity. It represents the

physiological ash and non-physiological ash. Physiological ash is the ash inherent in the plant due to biochemical processes and the non-physiological ash is due to contaminants from the environment. These may be carbonates, phosphates, nitrates, sulphates, chlorides and silicates of various metals which were taken up from the soil [28]. The non-physiological ash component of the total ash could be reduced by rinsing the fresh plant material several times in clean water before drying and processing for medicinal uses. The acid-insoluble ash value measures the amount of silica, especially siliceous earth, present in the crude plant [28]. The acid insoluble ash was 2.78% while the water soluble ash was 7.80% are within the range reported for some official vegetable drugs like *Cajanus* seed (not more than 0.15%), *Capsicum* fruits (not more than 1.5%), *Euphorbia* whole herb (3%) and *Atropa* leaf (5%) [28]. The value obtained for water soluble ash indicates that about 7.80% of the total ash of *T. diversifolia* will be physiologically available when the plant drug is ingested.

The moisture content of *T. diversifolia* leaves is 10.97% as obtained in the study which is similar to the value of 10.00% recorded for *T. diversifolia* leaves by [29] and higher than the value of 9.27% obtained by [24]. Moisture content value obtained in this study is within the acceptable limits of about 6 to 15% for most vegetable drugs [28]. The importance of low moisture content is that it reduces errors in the estimation of the actual weight of drug material, reduces components hydrolysis by reducing the activities of hydrolytic enzymes which may destroy the active components, and also reduces the proliferation of microbial colonies and therefore minimize the chance of spoilage due to microbial attack. Excessive moisture in a given sample encourages microbial growth or contamination [30], therefore we can say the understudied sample has limited chances of microbial invasion. The determination of the crude protein content of any sample is important because of their nutritive values and because protein has been proven to be of paramount importance for the survival of man [31]. *T. diversifolia* leaves shows a high Crude protein content of 27.89% which indicates that it can be a very good source of

protein. This value is almost double the value of 10.30% obtained by [29]. Proteins help improve the immune system and plays a major role in cell division and growth [32].

The study revealed Crude fibre content of 14.89% which is slightly higher than the value of 13.47% obtained for *T. diversifolia* leaves [24] and very much higher than 5.80% which was obtained by [29]. Fibres function by reducing the rate at which glucose is being absorbed into the blood stream thus reducing chances of having hyperglycemia [32]. Also, it is important to note that high fibre content of foods help in digestion and prevention of colon cancer [33]. This therefore implies that *T. diversifolia* has a potential to replace existing drugs used for the treatment of indigestion and colon cancer.

Fat composition was 1.14% which is quite low and is lower than the content of 2.47% reported by [24]. Fats are necessary for insulation and protection of vital organs as well as for hormone production [34]. The water-soluble extractive and alcohol soluble extractive gave 19.13% and 4.54% respectively. The result for water and alcohol soluble extractive indicates that the amount of phytoconstituent is more in water than in alcohol. Extractive values are useful to evaluate the chemical constituents in the crude drug, as well as to determine their solubility in a particular solvent (water or alcohol). The values obtained shows that water will be a better solvent for extraction of *T. diversifolia* than alcohol.

Elemental analysis (Table 3) revealed that *T. diversifolia* contains a high content of Magnesium (Mg) (6712.5mg/kg). The content of Mg is 22, 35, 122 & 144 times higher than the content of Fe (291.5mg/kg), Mn (191.0mg/kg), Zn (54.9mg/kg) and Cu (15.1mg/kg). This value of Mg is three (3) times higher than that that of *Cnicus benedictus* (1764.64ppm) [16], a member of Asteraceae family. The high content of Mg is very significant because Mg is an important mineral involved in various metabolic reactions [35] and it is necessary for major biological processes, for example, the synthesis of nucleic acids and proteins and it also plays a very important role in many physiological functions [36]. Mn and Fe content of *T. diversifolia* leaves, 191.00mg/g and 291.5mg/g falls within the same range with the

values 187.7mg/g and 261.2mg/g obtained for *Chromolaena odorata*, also a member of Asteraceae family while Cu and Zn content, (148.3mg/g; 95.0mg/g) of *C. odorata* is much higher than that of *T. diversifolia* (15.10mg/g; 54.90mg/g) [37]. Zn is an important mineral with antioxidant properties while Cu plays a major role in the utilization of Fe and mineralization of bones cortices. In Fe deficiency states known as anaemia, *T. diversifolia* leaves could be prescribed to ameliorate this condition [37]. Thus, we can infer that *T. diversifolia* can be used to treat and manage health conditions that set in due to the deficiency of some of these elements. However, elements (heavy metals) such as Ni, Co, Cr, Cd (toxic), Pb (toxic) were not detected in the plant. Reports suggest that there is a close relationship between cases of declining reproductive health and environmental pollutants like lead [38].

Also, it has been documented that metals such as lead, cadmium, arsenic and mercury can affect male reproductive functions including sperm motility and morphology [39] and spermatogenesis [40]. Thus, the absence or non-detection of these toxic heavy metals in this plant part further corroborates the safety of the plant.

5 Conclusion

In view of the increasing interest and demand for the use of medicinal plants for health management, there is the rising compulsory need for the standardization of these medicinal plants and this present study has helped to create these standards. This study has highlighted the phytochemical, proximate and elemental nature of *T. diversifolia* leaves. The presence of plant secondary metabolites such as tannins, flavonoids, saponins is indicative of its pharmacological potential since the presence of a particular secondary metabolite in any plant directly influences the therapeutic ability of the plant. Also, the proximate and elemental composition has helped to ascertain the safety, quality and purity profile of the understudied plant which are all quality indices necessary to achieve standardization. Thus, we can conveniently say that *T. diversifolia* leaves has a great potential for the management and treatment of diseases and information about the

plant has been documented therefore, it can serve as a substitute for the existing standard drugs used for the management and treatment of these diseases and health conditions. Worthy of note is the non-detection of toxic heavy metals in the understudied plant which has helped to further guarantee its safety. Also, this study has played a major contributory role in providing some of the plant information necessary for the development of monographs in herbal pharmacopoeias thereby serving as reference for future use. Since this study has reiterated the diverse potential of this plant as well as clearly highlighted its quality, it is therefore imperative for further studies such as *in vitro* and *in vivo* pharmacological assays to be carried out on the plant to help corroborate its specific activities.

6 Competing Interests

The author declared that no conflict of interest exists in this publication.

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