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PHYTOCHEMICAL AND PROXIMATE COMPOSITION OF *TITHONIA DIVERSIFOLIA* (HEMSL.) A. GRAY

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Abstract
Phytochemical and proximate composition of Tithonia diversifolia leaves, stem and root were compared in this study using standard procedures. The results showed that both aqueous and ethanol extracts of the plant parts tested positive for alkaloids, saponins, tannins, terpenoids flavonoids and phenols. These phytochemicals were found to be significantly highest in the leaves, followed by the root and the stem except the phenol which was significantly highest in the root. Among the phytochemicals, alkaloids was found to be highest (53.33 ± 6.58 mg/100 g) followed in decreasing order by tannins, flavonoids, saponins, terpenoids and phenols with respective mean values of 382.22 ± 7.58 mg/100 g, 338.89 ± 3.50 mg/100 g, 327.78 ± 7.33 mg/100 g, 65.00 ± 3.69 mg/100 g and 48.46 ± 0.32 mg/100 g. In all the proximate composition, crude fibre was highest (53.57 ± 0.14 %) and this was found in the stem, followed by carbohydrate (52.27 ± 0.32%) which was found in the leaves while the least proximate composition was crude fat (0.27 ± 0.03%) found in the stem. In all the plant parts, carbohydrate was found to be highest (41.84 ± 0.19), followed in decreasing order by crude fibre, moisture, total ash, crude protein and crude fat with mean values of 32.79 ± 0.10%, 9.19 ± 0.05%, 8.97 ± 0.07%, 5.99 ± 0.24% and 1.19 ± 0.05% respectively. The results indicated that, Tithonia diversifolia most importantly its leaves and root have high nutritive and medicinal values that could be explored for pharmaceutical purposes.

Keywords: *Alkaloids, Tannins, Flavonoids, Crude fibre, Carbohydrate, Tithonia diversifolia*

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1. INTRODUCTION

Tithonia diversifolia (Hemls.) A. Gray, a member of the Asteraceae family is an invasive perennial broad-leaved weed species reported to be introduced into West Africa as an ornamental plant (Akobundu and Agyakwa, 1997). The plant is about 2.5 m high, bushy and much branched. It reproduces from seeds and through vegetative re-growth of the basal stem when the plant is cut. The stem is quadrangular, spirally-ridged, pubescent below and glabrous above. The leaves are simple, alternate, lobed and of about 5-15 cm long and 3.5-6 cm broad. It is dark green, toothed and wedged shaped at the base (Akobundu and Agyakwa, 1997). The inflorescence is a solitary capitulum on a peduncle 7-15 cm long with large orange-yellow florets, while the fruit is compressed and about 6 mm long. In Nigeria, it emerges every year towards the end of rainy season and thrives well on a wide variety of ecological habitats ranging from sandy soil to loamy soils. It is commonly found growing in dense population in field grown crops, wastelands and most importantly on roadsides (Baruah et al., 2000). Locally, it is called *Agbale* among the Yorubas.

Analysis of solvent extracts of various plant species shows the presence of natural chemical substances such as alkaloids, tannins, phenolics, coumarins, terpenes, cyanates, glycosides, quinines and coumarins generally known as phytochemicals in plant species. Schuster et al. (1999) reported the isolation of sesquiterpene lactones from two *Tithonia* species: *Tithonia diversifolia* and *Tithonia rotundifolia* which was found to be pesticidal on test plants. *Tithonia diversifolia* has also been found to be highly medicinal on account of its antiplasmodial activity due to the presence of this sesquinterpene lactones and artemisinin acid analog (Ajaiyeoba et al., 2006). As an important medicinal plant, the leaf has been reported as the major organ used alone or in combination with other plants for

treatments of wide variety of ailments such as stomach pain, sore throat, indigestion, liver diseases and pains (Orwa et al., 2009).
Numerous literatures showed that most researchers focused on the phytochemical screening of *Tithonia diversifolia* leaves while ignoring its stem and root (Orwa et al, 2009; Olutobi and Olasupo, 2012). Aside from this, information on the proximate composition of the plant is also very scanty. It is therefore the attention of this study to compare the concentrations of both phytochemical and proximate composition of the leaves, stem and root of *T. diversifolia*

2. MATERIALS AND METHODS

Collection and preparation of plant samples

Fresh leaves, stem and root of *Tithonia diversifolia* were collected in November, 2013 from University of Ilorin, which is situated in the southern Guinea savanna zone of Nigeria (Latitude 8.5000^{o} N and Longitude 4.5500^{o} S). The voucher number UIH 586 was authenticated by the curator as deposited by the first author at the Herbarium of University of Ilorin, Ilorin. Nigeria. The plants were separated into leaves, stem and root and washed with distilled water to remove foreign particles. The plant parts were air dried under shade for 5 days after which they were ground into fine powder with the use of laboratory mill and then sieved using 2.00 mm wire mesh. The powder was then used for the extraction of the phytochemicals. The phytochemical and proximate analyses of the extracts were subsequently carried out at Kappa Biotechnology Laboratories, Ibadan. Nigeria.

Extraction procedure

The aqueous extract of the dried powder were obtained by soaking 20 g of each of the plant parts (leaves, stem and root) in 200 ml in distilled water at room temperature (27-28^{o}C) for 48 hrs. The extracts were filtered through Whatmann filter paper No. 42 (125 mm) and concentrated by gentle evaporation on a heating mantle (John-Dewole and Oni, 2013). The ethanol extract was done in similar manner as explained in the aqueous extract. The concentrated ethanol and aqueous extracts were then subjected to both qualitative and quantitative phytochemical screening. The solvent extractive values of leaves, stem and root of the test plant were also calculated.

Qualitative phytochemical examination

The aqueous and ethanolic extracts of leaves, stem and root were subjected to qualitative phytochemical analysis to test for the presence of secondary metabolites following the standard procedures of Ayoola et al. (2008).

Quantitative phytochemical examination

Quantitative phytochemical examination carried out on the aqueous and ethanol extracts of leaves, stem and root of *T. diversifolia* (Hemsl.) A. Gray were done using standard procedures (Onwuka, 2005). Phytochemical constituents determined were alkaloids, flavonoids, saponins, tannins, phenols terpenoids and phenols each in milligram per 100 g of the sample. Each of the constituents was done in triplicate for each of the plant parts.

Proximate composition

The proximate composition of *T. diversifolia* leaves, stem and root with respect to moisture, ash, crude fibre, crude protein, crude fat and carbohydrate were determined following the standard methods of Association of Official Analytical Chemists (AOAC, 2000).

Data analysis

Data were analyzed using Statistical Package for Social Science (SPSS) software version 17. Significant differences among the plant parts (leaves, stem and root) from means of three replicates were separated using Duncan Multiple range Test (DMRT) at 5% level of probability.

3. RESULTS AND DISCUSSION

3.1 Results

Ethanol solvent had the higher extractive efficacy for all the plant parts than aqueous

solvent with the exclusion of the stem. Percentage solvent extractive values regardless of the solvents were highest for root followed by the leaves and stem (Table 1).

Table 1: Solvent extractive values of *Tithonia diversifolia*

Plant parts	Solvent (%)	
	Ethanolic	Aqueous
Leaf	1.4	1.3
Stem	0.8	0.9
Root	2.1	1.8

Table 2 shows the results of qualitative analyses of *T. diversifolia* leaves, stem and root dry samples. In both solvents, there were presence of alkaloids, saponins, tannins, terpenoids flavonoids and phenols in all the plant parts (Table 2).

Data regarding quantitative analyses of *T. diversifolia* leaves, stem and root in the dried samples are presented in Table 3. Significant differences ($p<0.05$) were observed in all the phytochemical constituents among various parts of the plant. Phytochemical constituents such as alkaloids, tannins, saponins, flavonoids and terpernoids were significantly highest in leaves compared to those from the stem and root (Table 3). Phenols were found to be significantly highest in roots compared to those from leaves and stem. Significantly lowest values of all the phytochemical compounds were recorded in the stem when compared to other plant parts (Table 3). Regardless of the plant parts, alkaloids was found to be highest among all the phytochemical constituents with mean value of 853.33 mg/100 g and followed in decreasing order of tannins, flavonoids, saponins, terpenoids and phenols with respective mean values of 382.22 mg/100 g, 338.89 mg/100 g, 327.76 mg/100 g, 65.00 mg/100 g and 48.46 mg/100 g.

The results of proximate composition of *T. diversifolia* were presented in Table 4. The percentage moisture, ash, crude fibre, protein, crude fat and carbohydrate content of *T. diversifolia* ranged from 8.87-9.43%, 6.27-10.53%, 13.43-53.57%, 0.27-2.47%, 0.80-14.43% and 30.13-52.27% respectively (Table 4). Percentage moisture contents of *T. diversifolia* in root and leaves were statistically similar but significantly higher than that of the stem (Table 4). Significantly highest ash content was recorded in root and followed in decreasing order by leaves and stem. Percentage crude fibre showed similar trend of results as that of ash content except that significantly highest crude fibre was recorded in stem compared to those recorded from root and stem (Table 4). The results of crude fat, protein and carbohydrate showed similar pattern in that significantly highest values of these parameters were recorded in leaves and followed in decreasing order by root and stem (Table 4). Aside from the crude fibre, significantly lowest values of all the proximate composition of *T. diversifolia* were recorded in stem (Table 4). Among the proximate parameters, regardless of the plant parts, carbohydrate was found to be highest with mean values of 41.85%, followed by crude fibre (32.79%), moisture (9.19%), total ash (8.97%), crude protein (5.99%) and crude fat (1.19%) (Table 4)

Table 2: Qualitative phytochemical screening of ethanolic and aqueous extracts of *Tithonia diversifolia* leaves, stem and root.

Chemical Constituents	Ethanolic			Aqueous		
	Leaf	Stem	Root	Leaf	Stem	Root
Alkaloids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Phenols	+	+	+	+	+	+

Key: + = Present; - = Absent

Table 3: Quantitative phytochemical screening of *Tithonia diversifolia*

Constituent (mg/100 g)	Part				
	Leaf	Stem	Root	Mean	p-value
Alkaloids	1535.00 ± 10.41[a]	361.67 ± 3.33[c]	863.33 ± 6.01[b]	853.33± 6.58	<0.001
Tannins	540.00 ± 12.58[a]	125.00 ± 2.89[c]	481.67 ± 7.26[b]	382.22± 7.58	<0.001
Flavonoids	851.67 ± 4.41[a]	33.33 ± 1.67[c]	131.67 ± 4.41[b]	338.89± 3.50	<0.001
Saponins	761.67±14.24[a]	38.33±3.33[c]	183.33± 4.41[b]	327.78± 7.33	<0.001
Terpenoids	126.67 ± 4.41[a]	18.33 ± 1.67[c]	50.00 ± 5.00[b]	65.00 ± 3.69	<0.001
Phenols	64.58 ± 0.38[b]	9.77 ± 0.09[c]	71.03 ± 0.50[a]	48.46 ± 0.32	<0.001

Values followed by the same superscript along the row statistically the same at $p \leq 0.05$.
Values are presented as mean ± SEM
n=3 plant parts used (leaf, stem and root)

Table 4: Proximate composition of *T. diversifolia*

Proximate composition (%)	Part				
	Leaf	Stem	Root	Mean	p-value
Moisture	9.27 ± 0.09[b]	8.87 ± 0.03[c]	9.43 ± 0.03[b]	9.19 ± 0.05	0.001
Total ash	8.10 ± 0.10[b]	6.27 ± 0.03[c]	12.53 ± 0.09[a]	8.97 ± 0.07	<0.001
Crude fibre	13.47 ± 0.07[c]	53.57 ± 0.14[a]	31.33 ± 0.09[b]	32.79 ± 0.10	<0.001
Crude fat	2.47 ± 0.09[a]	0.27 ±0.03[c]	0.83 ± 0.03[b]	1.19 ± 0.05	<0.001
Crude protein	14.43 ± 0.12[a]	0.80 ± 0.58[c]	2.73 ± 0.03[b]	5.99 ± 0.24	<0.001
Carbohydrate	52.27 ± 0.32[a]	30.13 ± 0.09[c]	43.13 ± 0.15[b]	41.84 ± 0.19	<0.001

Values followed by the same superscript along the row statistically the same at $p \leq 0.05$.
Values are presented as mean ± SEM
n=3 plant parts used (leaf, stem and root)

3.2 DISCUSSION

The present investigation showed that both the ethanol and aqueous extracts of leaves, stem and root of *T. diversifolia* contain alkaloids, tannins, flavonoids, saponins, terpenoids and phenols. With respect to *Tithonia diversifolia* leaves, the results agreed with the findings of Olutobi and Olasupo (2012) who reported the presence of phytochemical compounds such as alkaloids, saponins, glycosides, flavonoids, tannins, terpenoids and phenols in the methanolic extract of *T. diversifolia* leaves. The quantitative occurrences of these bioactive substances except for phenols were significantly highest in the leaves compared to the stem and root. This could account for the reasons why the leaves has always been major organ used either alone or in combination with other plants for the treatment of many ailments such as stomach pain, sore throat, indigestion, liver diseases and pains (Orwa et al., 2009; Ezeonwumelu et al., 2012).

As observed in this study, alkaloids were found to be most abundant among the phytochemical constituents that were screened for and concentrated in the leaves and root. Alkaloids functions in the defense of plants against herbivores and pathogens, and are widely exploited as pharmaceuticals, stimulants, narcotics, and poisons due to their potent biological activities (Madziga et al., 2010; Doughari, 2012). Tannins, which were found to be next abundant to alkaloids, have been reported to be widely distributed in plant flora.

They are useful in wood healing (Kar, 2007) as astringents and antimicrobial (Singhal, 2001).
It is interesting to note that *T. diversifolia* leaves has abundant of flavonoids next to alkaloids but next to tannins in all the plant parts. Flavonoids are widely distributed among plant flora and numerous reports support their use as antioxidants or free radical scavengers as well as quenchers of singlet oxygen formation (Kar, 2007; Ali and Neda, 2011). Saponins were found to be concentrated in the root of the plant and have been found to be important therapeutically as they have been shown to have hypolipidemic and anticancer activity (Sarker and Nahar, 2007).
Terpenoids which is next to saponins have anti-inflammatory, sedative, insecticidal or cytotoxic activity, antimicrobial and neurotoxic action (Doughari, 2012). Also, they are major components of many essential oils (Martinez et al*.,* 2008). Phenols were found to be lowest among all the phytochemical compounds that were quantitatively determined, with highest concentration in root, followed by the leaves. Their role may be in plant defense against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections (Doughari, 2012).
The proximate composition of *T. diversifolia* leaves, stem and root showed significant difference ($p<0.05$) among the plant parts. Among the proximate composition, carbohydrate was found to be highest and concentrated in the leaves and root. The carbohydrate values of *T. diversifolia* which ranged from 30.13 ± 0.09-52.27 ± 0.32 % compared well with those of *Euphorbia heterophylla* (34.56 ± 1.6-65.65 ± 2.5%) as reported by Okeke and Adaku (2009). Carbohydrates are major source of energy for man and animals (Okeke et al., 2008). Crude fibre which is next abundant to carbohydrate was highly concentrated in the stem followed by the root. Dietary fibres perform a function of slowing down the rate of glucose absorption into bloodstream, thereby reducing the risk of hyperglycemia (Bouttwell, 1998; Okeke and Adaku, 2009). Dietary ash has proved helpful in establishing and maintaining acid-alkaline balance of the body system (Hawkkins, 1979). It also helps in the control of hyperglycemic condition in humans (Gokani et al., 1992). In this study, the ash content was found to be concentrated in leaves and root with values of 8.10 ± 0.10 and 12.53 ± 0.09 respectively.
Proteins were found to be concentrated in the leaves of *T. diversifolia*. Proteins boost the immune system and can play a role in cell division as well as growth (Okeke and Elekwa, 2006; Okeke et al., 2009). *T. diversifolia* is a poor source of fat. The crude fat content (0.27 ± 0.03-2.47 ± 0.09%) is low. Fats are necessary for hormone production, insulation and protection of vital organs (Dutta, 1981). Moisture content was higher in the root and stem compared to the leaves. Water is a universal solvent. It performs a function of dissolving other substances, carries nutrients and various materials round the body, making it possible for every of organ of the body to perform its functions optimally (Okeke and Adaku, 2009).

4. CONCLUSION

The present investigation show that variation exists in the concentration of both phytochemical and proximate constituents of *T. diversifolia* leaves, stem and root. Furthermore, most of these constituents were found to be abundant in the leaves and root but lowest in the stem. The presence of these phytochemical and nutrients indicates that *T. diversifolia* most importantly its root and leaves could serve as basic ingredient in drug making for use in treatment of diseases.

5. REFERENCES

[1] Ajaiyeoba EO, Abiodun OO, Falade MO, Ogbole NO, Ashidi JS, Happi CT, Akinboye DO (2006). *In vitro* cytotoxicity studies of 20 plants used in the Nigerian antimalarial ethnomedicine. Phytomedicine. 13(4): 295-298.
[2] Akobundu IO, Agyakwa CW (1997). A Handbook of West African Weeds. International Institute of Tropical Agriculture (I.I.T.A.) Ibadan, Nigeria. 18: 76 - 79.
[3] Ali G, Neda G (2011). Flavonoids and Phemolic acids: Role and biochemical activity in plants and

human. Journal of Medicinal Plants Research. 5(31): 6697-6703.
[4] AOAC. (2000). Official methods of Analysis (17th Edition). Volume 1. Association of Official Analytical Chemists. Inc., Maryland, USA.
[5] Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO (2008). Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. Tropical Journal of Pharmaceutical Research. 7(3): 1019-1024.
[6] Baruah NL, Sarma JC, Barua NC, Sarma S, Sharama RP (2000). Germination and Growth Inhibitory of Sesquiterpene Lactones and a Flavone from *T. diversifolia*. Phytochemistry. 36 (1): 29 – 36.
[7] Bouttwell RK (1998). An overview of the role of nutrition in carcinogenesis, nutrition, growth and cancer. Allan R. Liss Inc, London. 418 pp.
[8] Dalziel JM (1965). Useful Plants of West Tropical Africa. Bradford Publication, London. 804 pp.
[9] Doughari JH (2012). Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents. In: Rao, V. (Ed.), Phytochemicals- A Global Perspective of Their Role in Nutrition and Health, InTech, Shanghai. 538 pp.
[10] Dutta AC (1981). Botany for Degree Students, (6th edn). Oxford University Press, New Delhi. 708 pp.
[11] Ezeonwumelu JOC, Omolo RG, Ajayi AM, Agwu E, Tanayan JK, Adiukwu CP, Oyewale AA, Adzu B, Okoruwa AG, Ogbonnia SO (2012). Studies of Phytochemical Screening, Acute Toxicity and Anti-Diarrhoeal Effect of Aqueous Extract of Kenyan *Tithonia diversifolia* Leaves in Rats. British Journal of Pharmacology and Toxicology. 3(3): 127-134.
[12] Goffin E, Ziemons E, Mol P, Mdo CM, Martins AP, Cunha AP, Philippe G, Tits M, Angenot L, Frederich M (2002). *In vitro* antiplasmodial activity of *Tithonia diversifolia* and identification of its main active constituent: Tagitinin C Thieme. Journal of Plant Medicine. 68(6): 543-545.
[13] Gokani A, Ibrahim G, Shah H (1992). Alkaline-ash foods in the dietary management of diabetes mellitus. International Journal of Diabetes In Developing Countries. 12: 85-89.
[14] Hawkins HE (1979). Applied Nutrition, (2nd edn), Lee Foundation for Research, Milwukee, Wisconsin. 217 pp.
[15] John-Dewole OO, Oni SO (2013). Phytochemical and Antimicrobial Studies of Extracts from the Leaves of *Tithonia diversifolia* for Pharmaceutical Importance. Journal of Pharmacy and Biological Sciences. 6(4): 21-25.
[16] Kar A (2007). Pharmacognosy and Pharmacobiotechnology (Revised-Expanded Second Edition). New Age International Limited Publishers, New Delhi. pp. 332-600.
[17] Lockett CT, Calvert CC, Grivetti LE. (2000). Energy and Micronutrient composition of dietary and Medicinal wild plants consumed during drought: Study of Rural Fulani, Northeastern Nigeria. International Journal of Food Science and Nutrition. 51: 195-208.
[18] Madziga HA, Sanni S, Sandabe UK (2010). Phytochemical and Elemental Analysis of *Acalypha wilkesiana* Leaf. Journal of American Science. 6(11): 510-514.
[19] Martinez MJA, Lazaro RM, Del Olmo LMB, Benito PB (2008). Anti-infectious activity in the anthemideae tribe. In: Atta-ur (Ed.). Studies in Natural Products Chemistry, Vol. 35. Elsevier. pp. 445-516.
[20] Okeke CU, Adaku CN (2009). Phytochemical and Proximate Analyses of *Euphorbia heterophylla* Linn. (Euphorbiaceae). Nigerian Journal of Botany. 22(1): 215-222.
[21] Okeke CU, Elekwa I (2006). Proximate and Preliminary Phytochemical analyses of Avocado pear, *Persea gratissima* Gaertn. F. (Family Lauraceae). Nigerian Journal of Botany. 19(1): 156-162.
[22] Okeke CU, Izundu AI, Uzoechina E (2008). Phytochemical and Proximate study of female pawpaw (*Carica papaya* Linn.) Caricaceae. Journal of Science, Engineering and Technology. 15(2): 8207-8216.
[23] Olutobi O, Olasupo I (2012). Phytochemical Screening and the Phytotoxic Effect of Aqueous Extracts of *Tithonia diversifolia* (Hemsl) A. Gray. International Journal of Biology. 4(3): 97.
[24] Onwuka GI (2005). Food Analysis and Instrumentation: Theory and Practice. Naphthali Prints, Surulere, Lagos. 219 pp.
[25] Orwa CA, Mutua R, Kindt R, Jamnadass R, Simons A (2009). Agroforestry Database: A Tree Reference and Selection Guide Version 4.0. Available at http://www.worldagroforestry.org/resources/databases/agroforestree. (Accessed 28th February 2014).
[26] Sarker SD, Nahar L (2007). Chemistry for Pharmacy Students General, Organic and Natural Product Chemistry. John Wiley and Sons, England. pp. 283-359.
[27] Schuster A, Stokes S, Papastergious F, Castro V, Poveda L, Jakupovic J (1999). Sesquiterpene Lactones from two *Tithonia spp*. Journal of Phytochemistry. 31(9): 3139 – 3141.
[28] Singhal, A (2001). Options for non-surgical debridment of necrotic wounds. Advances in Skin Wound Care. 14: 96-100.
[29] Thompson LU (1994). Antioxidant and hormone-mediated health benefits of whole grains. Critical Review of Food Science and Nutrition. 34: 473-497.