ECOLOGICAL ANALYSIS OF THE PHYTOCHEMICALS IN STEM BARKS AND ROOTS OF *Calotropis procera (*R. BR.), AITON W. T. IN SOME STATES OF NIGERIA.

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Abstract

Analysis of the Phytochemicals of Calotropis procera in Ilorin and Gombe metropolises has been carried out. 20 freshly matured plants was sampled from each location the stem barks and roots were collected from East and West of the two metropolises, dried under shade and pulverised to powder using mortar and pestle, it was extracted using water, methanol and ethanol. The crude extracts was screened for Phytochemicals. Results revealed presences of alkaloids, flavonoids, saponins, tannin steroids, terpenoids, and glycosides in both selected parts with variations. Simple descriptive statistics was employed and further subjected to analysis of variance (ANOVA) to reveal the variability of the extractants or solvents used both within and between the metropolises. The results shows significant difference in all the extractants for stem barks and roots at ($\alpha = 0.05$) with p-values as 0.003, 0.020, 0.00 and 0.053, 0.005 and 0.000. for water, methanol and ethanol extracts respectively. A paired t-test was used to compare the Phytochemicals between the metropolises, and there was a significant difference at ($\alpha = 0.05$). For roots (0.277), and no Significant difference was observed in stem bark (0.002). The ethno botanical information collected revealed that Calotropis procera are commonly used as medicine, firewood, food and as fodder. The variability observed in the occurrence of the Phytochemicals might be attributed to the ability of the solvent to dissolve, differences in the age of the plant, and soil types in the study area.

Keywords: -Ecology, phytochemicals, extractants, extraction, shrub, solubility, variability.

Introduction

Stem barks and roots of plants including *Calotropis procera* are believed to have chemical constituents. Different parts of *C. procera* have been reported to exhibit ethno medicinal and nutritional properties while phytochemical evaluation of the plant parts revealed the presence of essential and trace elements in varied quantities.

C. procera belongs to the family Asclepiadaceae. It is commonly known as Apple of Sodom. It is an evergreen, erect and compact invasive shrub, up to 2.5 - 6.0 m tall in the dry savannah and other arid regions where it is much adapted to poor soils (Ismail 1992, Aliyu 2006). In Nigeria its vernacular names includes Bomubomu (Yoruba), Tumfafiya (Hausa) and Kayou (Kanuri) (Adoum *et al*. 1997, Noatay 2005). Two species represent this genus they are *C. procera* and *C. gigantea*. The main difference between the two is that *C. gigantea* has white flowers, while *C.*

procera has pinkish white flowers (Noatay 2005). Man since time immemorial has been using herbs or plants as medicine for developing immunity or resistance against cold, joint pains, fevers, etc. Medicinal properties are present in different parts of plants like root, stem, bark, heartwood, leaf, flower, fruits, seeds or plant exudates (Veerachari & Bopaiah 2011). This study was conducted in order to investigate effects of ecological types on the composition of Phychemicals in *C. procera* and its enthnobotanical used.

Materials and methods Study area description

Gombe metropolis is located in the north eastern part of Nigeria with a dry season climate commences from October and last till April and the rainy/wet from May – September with an average rainfall of 750 – 850 mm the area has Sudan Savanna vegetation. Ilorin is a city in Kwara State. It is located geopolitically in the North central of the country, the dry season commences from November to March and the wet/rainy from April to October, with an annual rainfall of 850 – 1,185 mm. The vegetation type is Guinea Savanna.

Plant collections and identification Study area

The stems and roots of a matured fresh plant of *Calotropis procera* were collected by carefully digging the soil with a hoe to obtain the roots and by using a knife to cut some branches of stems at Road Transport Ero'omo Park along Murtala Mohammed road Offa Garage (Ilorin East) and also at the premises of JADES Ventures Ltd near Kwara State Television, Apata Yakuba old Jebba Road (Ilorin West) which are on Latitude and longitude $8^{o}49$ N, $4^{o}65$ E respectively within Ilorin metropolis. Likewise same parts of this plant were also collected at Liji along Maiduguri express Road (Gombe East) and old mile 3 opposite Jiyamere Hotel alongside main Bauchi Gombe express Road, (Gombe West) on latitude and longitude, $10^{o}15$ N, $11^{o}10$ E respectively within Gombe metropolis. The freshly collected plant samples and pictures were taken to the University of Ilorin for identification, they were confirmed and authenticated at the herbarium of the Department of Plant Biology University of Ilorin, Kwara State.

Experimental design

Enough Stem barks and roots from a matured *C. procera* plants without physical signs of predation or pathogen attack were selected and collected. Samples collection was done during Raining Season May - July at the early hours of 7:20 – 10:20am morning. The stem barks and roots were washed with distilled water in the laboratory to remove impurities and dried under shade, they were then pulverized into coarse powder using mortar and pestle. The powdered samples were sieved and stored in an airtight container of capacity 1000 mL until when required for the Phytochemicals analysis.

Laboratory extraction of plant materials

This was carried out in the Biochemistry laboratory Gombe State University. 100 g of pulverized stem bark was weighed on an electrical balance with model number aeADAM RS-232. Soxhlet extractor (continuous hot extraction) was used. The pulverized (powdered) stem bark powder was taken in a thimble which was placed in a soxhlet extractor. The extractor containing the thimble and the stem bark powder was filled on top of the round bottom flask 500 mL and placed on the heating mantle. A condenser was fitted at the top of the extractor and a 99% concentration for each of methanol and ethanol was used, out of which 250 mL for each were measured and poured into the flask on the heating mantle. On heating, the solvent of (methanol and ethanol) evaporated to the condenser, where it condensed and trickled (drained) back to the extractor holding the thimble full with the plant material.

The extractor became full with the hot solvent it siphoned down to the flask along with the constituents. The recycling of the evaporated solvent was allowed until the extraction was completed. The extracts, was then poured into a different volumetric flask appropriately labelled and stored at room temperature before analysis. Same procedure was repeated for roots, using methods of extraction as described by Vishwa (2014). A cold extraction of the stem barks and roots in distilled water was performed where the samples was soaked in water for seven days after which the mixture was filtered with No. 1 filter paper, the extracts was also poured into a different volumetric flask appropriately labelled and stored at room temperature before analysis.

Collection of ethno botanical information

For collection of ethno botanical information the procedures of Alexiades (1996) and Cunningham (2001) was employed. The research was conducted in three phases. In first phase household surveys were conducted in 3 selected Gombe and Ilorin villages. The selection of the household was done at random where ten houses were counted and the eleven one picked as one, until ten household were selected. Only the male heads of the family were interviewed in 10 households. The respondents included city dwellers, village farmers and Cattle rearers. The ages of respondents were from 35 to 60 years. In the second step, local medicinal plant experts were selected by adopting method of Azhar *et al.* (2014) and individual interviews were conducted with 10 medicinal plant experts. A questionnaire was used which entailed major ethno botanical issues like common use, medicinal use and other modes of plant use. In third phase, stem barks and roots were sampled from the study area was dried under shade, ground and stored in an airtight container of capacity 1000 mL for analysis.

Statistical analysis.

The occurrence of phytochemicals were analysed. The qualitative data obtained were transformed to quantitative data by coding as, +++ represent 3.0, ++ represent 2.0, + represent 1.0 and finally – represent 0.0 for the purpose of statistical analysis. Analysis of variance (ANOVA) was used to obtain the variation among the phytochemicals and the extractants solvent used within and between the states, the means and p-value of the data were computed and then finally, the samples from the two states were compared using paired t – test.

Results

Table 1, Shows the qualitative results of phytochemical screening of stem bark collected from Ilorin and Gombe metropolises.

In Ilorin metropolis, crude extracts of stem barks water for phytochemical screening was found to be highly present with some little detection of the Phytochemicals in Ilorin East and Ilorin West, with non-detection of tannins in water extract. Similarly, in methanol crude extracts for the phytochemicals shows a similar trend with the same non-detection of tannins at Ilorin East and West respectively. Conversely, the ethanol crude extracts of Phytochemicals were found to be different in the Phytochemicals detection within location with a high to moderately presences with some traces detection of phytochemicals. Where some of these phytochemicals were not detected steroids, tannins, terpenoids in Ilorin East and glycosides, in Ilorin West respectively.

Table 1 results further revealed that the presence of Phytochemicals across location was found to be different within Gombe metropolis. Crude extracts for Water of the Phytochemicals was found to be moderate and highly present with some traces detection of Phytochemicals in Gombe west. Tannin, steroids and terpenoids were totally not detected in Gombe East and West respectively. Similarly, in methanol crude extracts for the phytochemicals screening it shows a similar trend with moderate and highly present with some little traces. But in both locations of Gombe metropolis East and West, a non-detection of saponins, glycosides and tannins were observed. Likewise, for ethanol crude extracts shows a similar trend with a moderate to highly presence of Phytochemicals detection in Gombe East and West. Steroids, tannins and terpenoids were completely not detected in both East and West of Gombe metropolis.

Comparison of the results between the States revealed that there was significant difference in terms of the presence of Phytochemicals in the extracts between different locations.

Table 1: Phytochemical screening of stem bark of *Calotropis procera* Collected from Ilorin and Gombe Metropolises

Phytochamicals	**Solvents/Extractants**											
	Water/Location				Methanol/Location				Ethanol/Location			
	IL-EST	IL-WS	GM-EST	GM-WS	IL-EST	IL-WS	GM-EST	GM-WS	IL-EST	IL-WS	GM-EST	GM-WS
Alkaloids	+++	+++	+++	++	+++	+++	+++	++	+++	+++	+++	+++
Flavonoids	+++	+++	++	++	+++	+++	-	+	++	+	++	++
Saponnins	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++
Steroids	+++	+++	++	-	++	+++	+	+	-	+	-	-
Glycosides	++	++	++	+	++	++	-	-	++	-	++	++
Tannins	-	-	-	+	-	-	-	++	-	+	-	-
Terpenoids	+++	++	++	-	+	+++	+	+	-	+	-	-

Key: IL-EST = Ilorin East, IL-WS= Ilorin West, GM-EST=Gombe East, GM-WS= Gombe West. +++ = highly present, ++ = moderately present, + = traces, - = not present

Table 2 shows the qualitative results of phytochemical screening of roots Collected from Ilorin and Gombe Metropolises.

The presences of Phytochemicals from the roots extracts were found to be different across location within the metropolis and between the metropolises.

In Kwara State, water extracts of the roots was found to be highly to moderate present with some little occurrence of the Phytochemicals in Ilorin West. Tannins, steroids and terpenoids were not detected in roots extracts in Ilorin East and in West that is in water extracts. Conversely, methanol extracts for the Phytochemicals shows a different trends with moderate to high presence with some trace amount in Ilorin West. But it was absent in glycosides, tannins and tannins at Ilorin East and West respectively. Moreover, the presences of Phytochemicals in ethanol extracts were found to be similar with methanol extracts, moderate to high present of the Phytochemicals, with trace amount observed at Ilorin east. Flavonoids, steroids, terpenoids were both absent within location and as well as tannins at Ilorin West.

The results from table 2 further revealed that the presence of Phytochemicals across location was found to be different within Gombe metropolis. Water extracts was found to be highly to moderate present of Phytochemicals in both locations, with trace amount of Phytochemicals and it was absent in flavonoids, glycosides, tannins, steroids, glycosides and terpenoids in both locations. Similarly, the presence of Phytochemicals in

methanol extracts shows a different pattern with moderate to high present with trace amounts of Phytochemicals at Gombe West, and it was absent for tannins in both East and West of the metropolis. Also, the presence of Phytochemicals in ethanol extracts shows a similar trend with moderate to high presence of Phytochemicals in both Gombe East and West, with trace amount. And it was totally absent for flavonoids, steroids, terpenoids, steroids and terpenoids at Gombe East and West respectively.

The comparison between the two metropolises, revealed that there was no significant difference in terms of the presence or occurrence of Phytochemicals in the roots extracts in the studied locations.

Table 2. Phytochemical screening of roots of *Calotropis procera* Collected from Ilorin and Gombe Metropolises

Phytochemical	**Solvents /Extractants**											
	Water/Location				Methanol/Location				Ethanol/Location			
	IL-EST	IL-WS	GM-EST	GM-WS	IL-EST	IL-WS	GM-EST	GM-WS	IL-EST	IL-WS	GM-EST	GM-WS
Alkaloids	+++	++	+++	+++	+++	+++	+++	+	+	++	+	++
Flavonoids	+++	+++	-	+	++	++	++	++	-	-	-	+
Saponins	+++	+++	+++	++	+++	+	+++	++	+++	+++	++	+
Steroids	+++	-	+	-	++	+	++	+	-	-	-	-
Glycosides	+++	+	-	-	-	++	+++	+	++	++	+++	+
Tannins	-	+	-	+	-	-	-	-	+	-	+	++
Terpenoids	+++	-	+	-	++	+	++	+	-	-	-	-

Key: IL-EST = Ilorin East, IL-WS= Ilorin West, GM-EST=Gombe East, GM-WS= Gombe West. +++ = highly present, ++ = moderately present, + = traces, - = not present

Table 3. One-Way ANOVA of the solvent extracts for stem barks and roots of *Calotropis procera* within and between the two metropolises.

The presences of Phytochemicals in the water extracts of the stem barks of *Calotropis procera* within and between the metropolises shows that there was significant difference within and between metropolises in the occurrence of the Phytochemicals in the stem barks in water extracts with p-value (0.003) against alpha ($\alpha = 0.05$).

Similarly, the presences of Phytochemicals in methanol extract of the stem barks shows that there was no significant difference

among and between the groups in the two metropolises with p-value (0.020) against the alpha ($\alpha = 0.05$).

In the same trend, the presence of Phytochemicals in ethanol extracts shows no significant difference in the occurrence of the Phytochemicals in the stem barks of *Calotropis procera* within and between the metropolises with p-value (0.000) against the alpha ($\alpha = 0.05$).

The presence of Phytochemicals in water extracts of the roots of *Calotropis procera* within and between the metropolises shows that there was no significant difference within and between the metropolises in the occurrence of the Phytochemicals found in the roots of *Calotropis procera* in water extracts with p-value (0.053) against alpha ($\alpha = 0.05$).

Similarly, the presence of Phytochemicals in methanol extracts of the roots shows that there was no significant difference among and between the groups in the two metropolises with p-value (0.005) against the alpha ($\alpha = 0.05$).

In the same trend, the presence of Phytochemicals in ethanol extracts shows no significant difference in the occurrence of Phytochemicals found in the roots of *Calotropis procera* within and between the metropolises with p-value (0.000) against the alpha ($\alpha = 0.05$).

Table 3. One-Way ANOVA of water, methanol and ethanol extracts of the stem barks and roots of *Calotropis procera* from the two metropolises.

Solvent extract difference within and between the states	Stem barks		Roots	
	F-value	p-value	F-value	p-value
Water	5.000	.003	2.532	.053
Methanol	3.275	.020	4.373	.005
Ethanol	16.167	.000	8.853	.000

Significant at ($p < 0.05$)

Table 4. T-test analysis to compare the Phytochemicals of *Calotropis procera* stem barks and roots between Ilorin and Gombe metropolises.

The results revealed the mean value of the stem barks as (1.9762) and (1.4048) and the mean difference as (0.91654) between the two metropolises. The t-value value was (3.344) with p-value as (0.082) indicating that there was a significant difference in the presence and occurrence of these Phytochemicals in the stem barks of *Calotropis procera* at alpha ($\alpha = 0.05$) level of probability.

The results revealed the mean value as (1.3333) and (1.1190) and the mean difference was (0.60687) between the two metropolises. The t-value was (1.102) with p-value as (0.277) indicating that there was a significant difference in the presence and occurrence of these Phytochemicals found in the root of *calatropis procera* at alpha ($\alpha = 0.05$) level of probability.

Table 4. Paired t-test of the stem barks and roots *Calotropis procera* between the two metropolises.

	Mean	**95% Confidence Interval of the Difference**	**T**	**Df**	**Sig. (2-tailed)**
STEM BARKS					
Ilorin	1.9762				
Gombe	1.4048				
Ilorin-Gombe		0.91654	3.344	41	0.082
ROOTS					
Ilorin	1.3333				
Gombe	1.1190				
Ilorin-Gombe		0.60687	1.102	41	0.277

Table 5. Common uses of the plant *Calotropis procera* according to different respondents in Gombe and Ilorin metropolises.

The results in Gombe villages revealed the different uses of *C. procera* shrub and the common multipurpose uses were divided into six categories i.e., medicinal, fodder, firewood, food, aesthetic and miscellaneous. Local dwellers respondents expressed their opinion about the uses of this plant as; food, fodder, medicinal and firewood 25.80%, 25.80%, 22.58%, and 22.58% respectively. And medicinal plant expert opined to the use of this plant as firewood 24.39%, fodder 21.95%, medicinal 21.95% and food 19.51% and a few of city dwellers' responded to this as firewood 31.57% and is medicinal 26.31%.

Also the results from Ilorin villages revealed a similar trend in the uses of *C. procera* shrub in Ilorin and its environs. Local dweller responded positively to the uses of this as; firewood 23.68%, fodder 21.05%, medicinal 26.31%, and food 18.42%. Likewise the medicinal plant expert responded in a similar trend that it is as; medicinal 22.72%, fodder 20.45%, food 18.18%, firewood 18.18% and the fruit silk is used in making pillows for neck pain relief. And city dwellers respondents opined to the uses of this plant as medicinal 21.08%, fodder 21.73% and food 30.43% for animals.

E.g. * = thatch roof houses, pillows, beds etc.

Table 5: Common plant *Calotropis procera* uses according to different respondents in Gombe and Ilorin metropolises.

Plant use category	Citation responses in (%)					
	Gombe			Ilorin		
	local dweller's	medicinal plant experts	city dweller's	local dweller's	medicinal plant experts	city dweller's
Firewood	25.80	24.39	31.57	23.68	18.18	17.39
Fodder	25.80	21.95	15.78	21.05	20.45	21.73
Medicinal	22.58	21.95	26.31	26.31	22.72	26.08
Food	22.58	19.51	21.05	18.42	18.18	30.43
Aesthetic	0.0	7.31	5.26	7.89	4.54	4.34
Miscellaneous*	3.22	4.87	0.0	2.63	15.90	0.0

Discussions

The phytochemicals occurrence in the crude extracts of water, methanol and ethanol extracts of stem barks, and roots of *Calotropis procera* when screened, revealed the presence of alkaloids, flavonoids, saponins, tannin steroids, terpenoids, and glycosides occurring in both selected plant parts (Table 1, 2). This finding was in agreement with the work of Ahmed *et al.* (2005), Ghias *et al*. (2012) and Amit *et al*. (2014). Who reported that the plant *C. procera* is a rich source of various Phytochemicals like; steroids, terpenoids, saponins and glycosides, flavonoids. It was also in agreement with (Jagan *et. al*. 2014). Who reported a similar investigations that the various extracts of *C. procera* for phyto-chemical analysis were found positive for saponins, flavonoids, tannins, alkaloid and glycosides occurring in these parts of the plant species.

Table 3. Revealed the presence of Phytochemicals in different extractants adopted for the extraction of the phytochemicals, using one-way analysis of variance (ANOVA) which shows that there was no significant difference in the occurrence of Phytochemicals in stem barks and roots of *C. procera* at ($\alpha = 0.05$). This finding was in harmony with (Mohammed & Dabai 2008). Who reported that seasonal variations can affects the chemical composition of the plants.

Table 4. Revealed the results for paired t-test of the phytochemical within and between the two metropolises. The plant stem barks indicated that there was a significant difference with t-value as (0.277) between

the two metropolises at (α = 0.05). Same trends was observed for roots (0.082) sampled between the two metropolises. These variations might be as a result of difference in the age of the plants randomly selected for the study. And also differences in geographical location, climate and also the vegetation types in the study area as Sudan and Guinea Savannah might be the reasons for these variations. This finding was in agreement with the work of (Ramachandran *et al.* 1980, Adoum *et al.*1997) who reported that the variability observed in the amounts of the elements or compounds obtained in the present study could be attributed to the variability in geographical location of the plants as well as the part of the plant examined.

However, the occurrence of the various classes of secondary metabolite varies amongst the extracts evaluated. These variation might be as a result of the type of phytochemicals present in different parts as shown in the results of phytochemical screening (Table 1, 2), also it might be attributed to the ability of the solvent to dissolve into solution specific type of phytochemicals as reported by Kawo (2007), Yusha'u *et al*. (2008) and Kawo *et al.* (2009).

Conversely, Kawo (2007) reported, the non-detection of flavonoids, glycosides, steroids, terpenoids and tannins of Phytochemicals in some extracts of *C. procera* plant screened in his study, and explained that such difference in the extracts yield might be attributed to the availability of different extractable components as well as to the nature of soil and agro-climatic conditions of the regions from where the plant tissues are harvested.

Time of collections of the different plant parts can also affect the phytochemicals effectiveness. A similar claim was reported by WHO (2003), that time of plant collection can affect the chemical composition of the plants Phytochemicals. Also (Adoum *et al*. 1997, Odugbemi 2008). Made a similar claim that the geographical location of a plant can affect its active constituents, which may be induced by many factors like climate, propagation method, time of collection of plant parts also affects its effectiveness.

Table 5 revealed the different uses of *C. procera* plant as a shrub in the two states. The respondents opined about medicinal, fodder, miscellaneous, food and firewood uses of this plant. The groups responded positively on the use of this plant as fodder, such that goat do grazed/feed on this plant during dry season, as medicine, as firewood such as the stems when collected and dried could be a source of firewood to local dwellers, so also as food, Fulani used the leaves to coagulate their milk to produce local cheese known as 'Wara' in Yoruba.

The ethno botanical study on *C. procera* shrub has revealed the great diversity of its popular medicinal uses and common uses for a wide range of ailments. Either as part or whole plant is used single or in combination with other plant materials or mineral to enhance its effectiveness and efficacy.

Conclusion

The study on the Ecological analysis of the phytochemical presence in stem barks and roots of *Calotropis procera* has been carried out and the results revealed the presences of phytochemicals such as alkaloids, flavonoids, saponins, steroids, glycosides, tannins and terpenoids with significant difference in their occurrence of the studied parts. The ethno botanical information on the use of *Calotropis procera* revealed that it can be used as

fodder, medicinal, firewood and food, which makes it an essential plant.

It is therefore, recommended that further research be carried out in order to isolate and purify the phytochemicals constituents of this *C. procera* using different solvent for the extraction techniques, with a view to justify these claims.

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