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An Investigation of Phytoconstituents, Cytotoxicity and Proximate Analysis of Some Medicinal Plants in Kashere, Gombe State - Nigeria

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Abstract

Extracts of *Sassafras albidum* (Lauraceae)*, Detarium microcarpum* (caesalpinioideae) and *Echinaceae angustifolia* DC (Compositae) were screened for the presence of secondary metabolites and Brine Shrimp Toxicity assay (BST). Dried powdered samples of the same plants were analyzed for their proximate composition. The results showed that most of the important secondary metabolites were present including alkaloids, tannins, flavonoids, saponins, phlabotannin, anthraquinone, resin, Terpenoid and glycosides. The result of the cytotoxicity screening showed that *E. angustifolia* was most toxic to the nauplii. LC50 values of the proximate composition of *Sassafras albidum* revealed 62.5% moisture content, 71.0% ash, 67.0% fiber, 13.33% fat, 0.79% protein, *Detarium microcarpum* has 2.0% moisture, 3.5% ash, 0.83% protein, 6.67% fat and 79% carbohydrate. *Echinaceae angustifolia* DC on the other hand showed 32.5% moisture, 93.0% ash, 10.0% fiber, 3.33% fat, 3.33% protein. The result of the cytotoxicity revealed LC50 of 63.1µg/ml 158.49 µg/ml 316.23 µg/ml for *Sassafras albidum, Detarium microcarpum,* and *Echinaceae angustifolia* DC respectively.

Keywords: Cytotoxicity; Medicinal Plants; Phytoconstituents; Proximate Analysis

Introduction

Plant kingdom is a treasure house of potential drugs and in recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are readily available, less expensive, safe and efficient and rarely have side effects. According to World Health Organization, medicinal plants would be the best source to obtain variety of drugs. Large percentage of people from developed countries uses traditional medicine, which has compounds derived from medicinal plants. However, such plants should be screened and studied better to understand their properties, safety, and efficiency.

However, most of the available information regarding the medicinal potential of these plants is not provided with credible scientific data. For this reason, several researches have been conducted to determine the toxicity of medicinal plants.

Many of today's drugs are derived from plant resources. Historically, medical plants have provided as a source of inspiration for novel therapeutic drugs, as plants derived medicines have made large contributions to the health and well-being of human beings. Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. *Echinaceae* is a genus of herbaceous flowering plants in the daisy family. It has ten species which are commonly called purple coneflowers; they are commonly found in eastern and central North America, where they are found growing in moist to dry land and open wooded area. They have large showy heads

and composite flowers and belongs to Asteraceae family, a family that is important to commerce for its medicinal and culinary herbs. The species of *Echinaceae* include; *Echinaceae angostifolia*-Narrow-leaf Coneflower, *Echinaceae atrorubens*-Topeka purple coneflower, *Echinaceae laevigata*-smooth coneflower, smooth purple coneflower, *Echinaceae pallida*-pale purple coneflower, *Echinaceae paradoxa*-bush's purple coneflower, yellow coneflower, *Echinaceae purpurea*-eastern purple coneflower, *Echinaceae sanguinea*-sanguine purple coneflower, *Echinaceae serotina*- narrow-leaved purple coneflower, *Echinaceae simulata*-wavy-leaf purple coneflower, *Echinaceae tennesseensis*-Tennessee coneflower, but only three are marked in the medicinal herb trade, they are *E. angustifolia, E. purpurea* and *E. pallida* [2].

Echinaceae angustifolia DC has alternative names like Kansas snakeroot, narrow-leaved purple coneflower, scurvy root, comb flower, black Susan and hedgehog.

The *Echinaceae angustifolia* (coneflower) till today is the most widely used medicinal plant of the plains India. It was used as pain killer and for a variety of ailment, which includes toothache, cough, cold, sore throat, infections like syphilis and septic wounds, even as an anti-toxin for snake bite and blood poisoning. The Cheyenne chewed it to stimulate the flow of saliva, which was useful for sun dance participants as a thirst preventive. This plant has a reputation for poor growth, but when the proper steps are followed, it has extremely dependable germination. The seed require 12-weeks period of cold conditioning to germinate [3].

In Nigeria *Detarium microcarpum* is locally called ofor by the Igbos, taura by the Hausas, and oghogho by the Yorubas in Nigeria, is a leguminous plant of the leguminosae of flowering plants and member of sub-family Caesalpiniaceae. It is found in both the rain forest zones and the drier savannah areas of Africa. *Detarium* microcarpum exists in Nigerian's geographical regions [4]. Among the igbos in south eastern part of Nigeria, it is mythically conceived to be a chip of the primal tree known as "Ofo" that grows in gods own compound, it symbolizes truth and honesty [5]. Nutritionally, the seed which is used as a traditional soup thickener contain lipids, carbohydrates, proteins, crude fiber and essential elements likes Na, K, Mg, Ca, S, P and Fe [6]. In African ethno-medicine, the plant and the related species *Detarium senegalense* are used in the treatment of syphilis, dysentery, bronchitis, leprosy, sore throat, pneumonia, diarrhea, malaria and meningitis [7]. The fruits of this lesser known legume are drupe - like, circular and disc shaped with a distinctive layer of greenish, meanly pulp that is tangled with numerous network of fibers. The seeds occur singly and are embedded within the fibrous network of the fruit. It is hard, disc shaped and wrinkled, brownish colored husks. The cotyledon is white in color. This legume has found important application as a soup thickener in parts of Abia, Imo and Anambra. Soup thickeners noted increase the palatability of the soup and reduce bulk in the diet. The thickening and the binding properties of the gum derivable from the ripped seeds have been extensively studied. One use being advocated is as a possible tablet binder. *Detarium microcarpum* is a small tree up to 10m tall, with horizontal root system. It is confined to drier regions of west and central Africa. In Burkina Faso, the fruit pulp of *D. microcarpum* is used to treat skin disease.

In Mali the bark is used to treat measles, itching, hypertension, tiredness while the decoction of the leaves or root is used for paralysis and cramp, meningitis, difficult in delivery and tiredness, inhaled smoke of leaves treats fever in cattle. In Niger and Togo, the fruit preparation is used to treat dizziness, while in Benin; the decoction of the leave is used in treating convulsion and fainting. Apart from medicinal uses, the fruit of *D. microcarpum*is sweet and commonly eating fresh, while the pulp is used in making couscous and cake as well as substitute for sugar. The seeds are used as frankincense to ward off evil spirits. The seeds and leaves are eating as condiments and vegetable [7]. *D. microcarpum* is an edible fruit enriched with vitamin C, the seeds and leaves are used for cooking, the roots, stem bark and stems are used for treatment of ailments like meningitis, tuberculosis, itches and diarrhea. *D. microcarpum* flowering period is rainy season; it inhibits Sudanese and Guinean Savannahs, dry forest, fallow land, from Senegal through Nigeria to Cameroon in West African, and as far as Sudan. The leaves of this plant are used as an organic fertilizer. The tree produces a fragment resin. The root is heated, and sweetly scented and are used as perfume and mosquito repellent. The seeds are used to make necklaces for women, in southern Mali the leaves are used as roofing material. The leaves are used to make mask. The wood is hard, moderately heavy and it's easy to work on, it is durable and last long even under water. The wood is used for carpentry, poles and fence. The wood is good to be used as firewood, as it lights quickly even if wet. These exotic trees can, however, be powerful sources of oil for nutritional, lipid-based and pharmaceutical industries. Ononogbu gave the percentage oil as 12% for *Detarium microcarpum* which compares favorably with that of *Zea mays* (maize) [8].

Sassafras albidum, is commonly known as white sassafras, red sassafras or silky sassafras. It is from the family of *Laurecea*, a native of north America. It is pyramidal when young but later develops into a 30-60 feet-tall by 25 to 40 foot-wide, round canopy, composed of many short, horizontal branches which gives the tree a layered effect. *Sassafras* was grown for the purpose of its medicinal properties. *Sassafras* is a fast growing, aromatic tree, with three distinctive leaf shapes. It grows largest in a smoky mountain on moist well drained sandy loam in open wood land. The flower of sassafras is small but quite snowy, both male and female are bright yellow-green borne in a 2inch recimes appearing in middle spring. The fruits are dark shiny blue, ovoid, fleshy drupes (1/3-inch-long) that are borne in a red cup attached to a red stalk, held upright maturing late summer. The twig is slender, green and sometimes

pubescent, with a spicy-sweet aroma when broken; the buds are ¼ inch long and green. The bark is brown with cinnamon-brown inner bark, becoming coarsely ridged and furrowed, when is cut, is brings out spicy aroma. The male flowers have nine stamens, the female flowers have six staminodes pollinated by insects. All parts of *Sassafras albidum* have been used for human purposes these parts include, stems, leaves, bark, wood, roots fruits and flowers.

Some tribes in America use the leaves treat wounds by rubbing the leaved directly on the wound and use different parts of the plant for many medicinal purposes, like acne, urinary disorder, high fever, they also use the bark as a dye and as flavoring agent. *Sassafras* wood is also used as a fire starter due to the flammability of the natural oil.

Materials and Method

Collection of Samples

The stem barks of the three plants were collected in Kashere, Akko Local Government Area, Gombe state, Nigeria. The stem barks were air-dried in the laboratory and ground to fine powder using mortar and pestle. The powdered samples were stored in polyethylene bags at ambient temperature until when needed for further analysis. The same plant samples from the same source and prepared the same way were used for the proximate analysis.

Reagents and Apparatus

All chemicals used were of analytical grade, these includes; methanol, ethanol, sulfuric acid(VI) (H_2SO_4), iron(III) chloride ($FeCl_3$), hydrochloric acid (HCl), carbon tetrachloride (CCl_4), sodium hydroxide (NaOH) ammonia (NH_3), copper(II) sulphate solution, chloroform ($CHCl_3$), Wagner's reagent, sodium nitrate(V) ($NaNO_3$), Potassium chlorate (V) ($KClO_3$), potassium heptaoxodichromate (VI) ($K_2Cr_2O_7$), and nitric acid (HNO_3), soxhelet extractor, beakers, spatula, test tubes, measuring cylinder, volumetric flask, nickel crucible, filter paper, weighing balance, furnace, oven, digester, water bath, thermometer.

Extraction

The powdered stem bark (200g) of each plant (*Detarium microcarpum, Echinacea angustifolia* DC, *Sassafras albidum*) were extracted with methanol. 50g of the powdered sample was packed in a thimble at a time and extracted at a temperature of 70^{o}C for 8hrs in four successive extractions using Soxhlet extractor. The solvent was recovered each time on a Rotary evaporator.

Phytochemical Screening

Standard method described by Abdu, et al. (2015) [9] was adopted to test for the presence of some important phytoconstituents.

Cytotoxicity (BST) Assay

Artemia salina eggs were added into a hatching chamber ¾ filled with ocean sea water. The chamber was kept in an open space for 24 hours, after which the eggs hatched into shrimp larvae. 4 mL ocean water was added and ten (10) larvae of *Artemia salina* were introduced into each vial. After 24 hours of introducing larvae, the number of survivals were counted in triplicate and recorded. To each sample vial, a drop of DMSO solvent was added, ten shrimps were transferred using a Pasteur pipette, and artificial seawater was added to make a total volume of 5 ml. The nauplii were counted against a lighted background. Counting for the chronic LC50 began 24 hours after initiation of tests. Nauplii were considered dead if they were lying immobile at the bottom of the vials, and the percentage of deaths at each dose and at the control were determined [10].

Proximate Analysis

The proximate analysis was carried out in triplicates and the results obtained were the average values, the estimation of the various food parameters in the sample were determined by using modified Uzama (2012). The carbohydrate was determined by difference method, i.e. 100 - (fat + protein + moisture + ash). The nitrogen which is the precursor for protein of a substance was determined by micro- kjeldahl method. The moisture and ash were determined using weight difference method. Protein, Carbohydrate, Fiber, Fat, Ash, and Moisture were analyzed and results presented in (Table 3).

Determination of Protein

The dried ground sample weighed 0.5g was placed in a Kjaldahl flask to which 18.0 ml of H_2SO_4, 1.0g of $CuSO_4$, 25ml of conc. H_2SO_4 were added and digested for 6hrs. The mixture was cooled down to room temperature. It was transferred to about 50ml of 4% boric acid solution in a receiving flask and 5 drops of mixed indicator was add to it and placed under the condenser of the Kjaldahl distillation unit. 50ml of water and 60ml of 32% of NaOH solution were added to the Kjldahl flask and distilled so that a volume of 200ml was collected in the receiving flask. 0.1m Hcl was placed in a burette and titrated against the flask content. % protein is given by:

% protein = % Nitrogen x 6.25.

Determination of fiber

Each of the sample weighing (0.1g) were transferred into a clean crucible to which three drops of octanol were added to prevent foaming and heated to boiling for 30mins. It was filtered and washed three times with hot water from spray device and dried thoroughly. 150ml of KOH solution was added to each sample followed by few drops of octanol and boiled for another period of

30mins. The percentage crude fiber was obtained as followed:

% crude fiber =$W1 - W2/W0$ X 100.

Determination of Fat Content

The total fat was extracted with petroleum ether using Soxhlet extractor. To determine the percentage of fat, 2.0g of the dried plant part was extracted with 1 L of petroleum ether. The plant part powder was dried and the percent loss of weight was calculated.

Determination of Ash Content

A cleaned flat bottomed silica dish was held in a hot burner flame for 1minute and transferred to a desiccator then cooled, weighed and labelled. (W). 5.0g of plant sample was weighed into a dish (W_1) and heated gently then transfered to a muffle furnace at 550^{0}C. The heating was maintained until the carbon has burnt away. It was weighed and labeled as (W_2). The ash content was obtained as followed:

Weight of the empty dish = W

Weight of empty dish + sample = W_1

Weight of empty dish + ash = W_2

% ash = $\frac{W1-W2}{Wt\ of\ Sample}$ X 100 [11].

Determination of Moisture Content

One gram of the powdered sample was weighed in a clean beaker of known weight. The sample was then dried in oven at 105°C for 8hrs. The beaker was cooled and weighted to determine water loss in powdered sample.

Determination of Carbohydrate Content

Carbohydrate was determined by subtracting the weights of crude protein, crude fats, crude fiber, ash, and moisture content from 100. % carbohydrate = (100 -protein + ash + fiber + fat + moisture).

Results and Discussion

S.No	Plant	Mass (gm)	% Yield
1.	*Sassafras albidum, (Lauraceae)*	200	19.5
2.	*Echinaceae angustifolia* DC (Compositae)	200	17.0
3.	*Detarium microcarpum* (caesalpinioideae)	200	37.4

Table 1: Percentage Yield of Extracts.

Phytoconstituent	*Sassafras albidum,*	*Detarium microcarpum*	*Echinaceae angustifolia* DC
Alkaloid	++	++	+++
Terpenoid	+++	+	++
Sapponnin	++	-	++
Glycoside	+++	+	+
Resin	-	++	-
Phlabotannin	-	-	-
Steroids	+++	+++	++
Flavonoid	++	++	+++
Anthraquinone	+	-	+
Key: Low (+), High (++), Very High (+++), Not Detected (-)			

Table 2: Phytochemical Screening Result of Methanol Extract of *Sassafras albidum, Detarium microcarpum* and *Echinaceae angustifolia* DC.

Conc. (µg/ml)	Survivals			Deaths			% mortality	Log10Conc.	LC50(µg/ml)
	V1	V2	V3	V1	V2	V3			
1000	1	3	3	9	7	7	76.9	3	316.23
500	5	5	3	5	5	7	56.67	2.7	
250	8	8	7	2	2	3	23.33	2.4	
125	8	7	9	2	3	1	19.33	2.1	
62.5	9	10	8	1	0	2	10.9	1.8	
+ctrl	0	0	0	10	10	10	100	-	
-ctrl	10	10	10	0	0	0	0.00	-	

Table 3: BST Assay Results of MeOH extract of Stem bark of *Echinaceae angustifolia.*

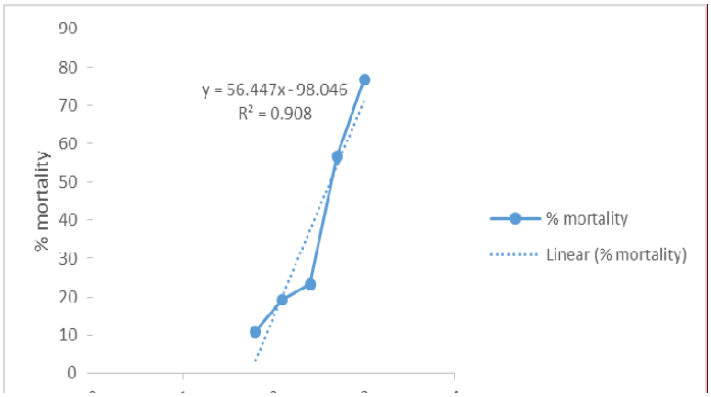

Figure: 1 Cytotoxicity graph of % mortality versus log10 Conc. For Stem bark extract of *Echinaceae angustifolia.*

Conc. (µg/ml)	Survivals			Deaths			% mortality	Log10Conc.	LC50(µg/ml)
	V1	V2	V3	V1	V2	V3			
1000	0	0	3	10	10	7	90.0	3	
500	2	3	2	8	7	8	76.67	2.7	
250	4	3	3	6	7	7	66.67	2.4	
125	6	7	7	4	3	3	33.33	2.1	158.49
62.5	9	10	8	1	0	2	10.0	1.8	
+ctrl	0	0	0	10	10	10	100	-	
-ctrl	10	10	10	0	0	0	0.00	-	

Table 4: BST Assay Results of MeOH extract of Stem bark of *Detarium microcarpum.*

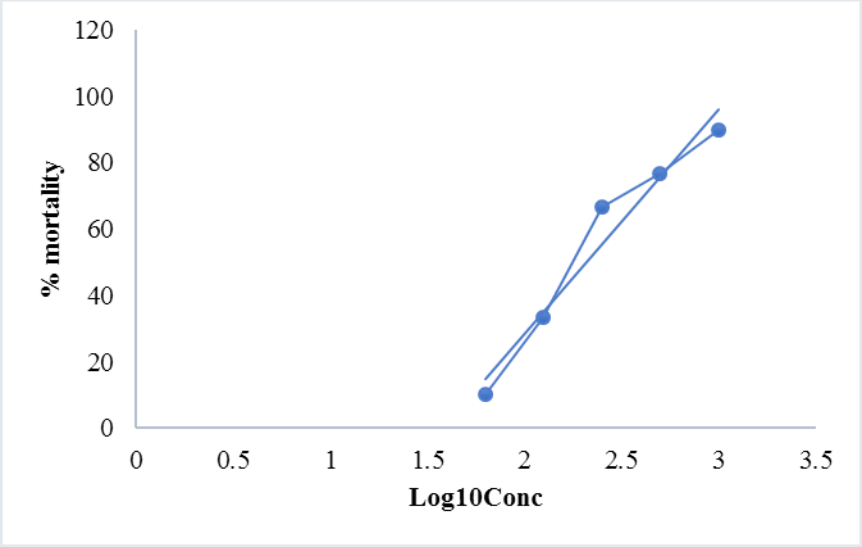

Figure 2: Cytotoxicity graph of % mortality versus log10 Conc. For Stem bark extract of *D. microcarpum.*

Conc. (µg/ml)	Survivals			Deaths			% mortality	Log10Conc.	LC50(µg/ml)
	V1	V2	V3	V1	V2	V3			
1000	0	3	2	10	7	8	83.33	3	63.1
500	2	4	2	8	6	8	73.33	2.7	
250	3	3	3	7	7	7	70.0	2.4	
125	2	4	5	8	6	5	63.33	2.1	
62.5	5	8	4	5	2	6	43.33	1.8	
+ctrl	0	0	0	10	10	10	100	-	
-ctrl	10	10	10	0	0	0	0.00	-	

Table 5: BST Assay Results of MeOH extract of *Sassafras albidum.*

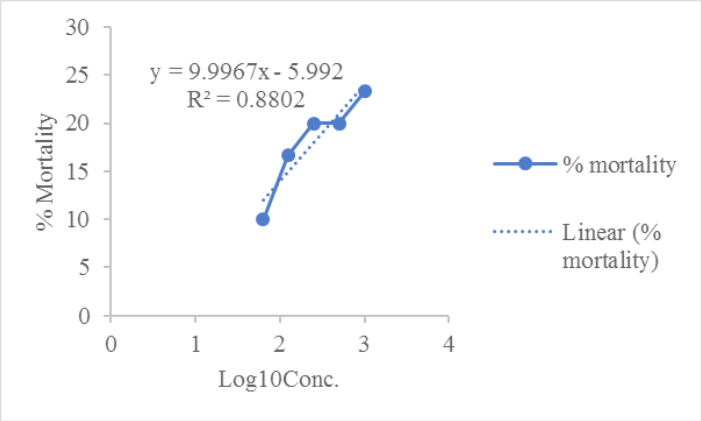

Figure 3: Cytotoxicity graph of % mortality versus log10 Conc. for *S. albidum extract.*

Composition	*Detarium microcarpum*	*Sassafrass albium*	*Echinacea angustifolia*
Moisture	0.20±0.03	0.63±0.01	0.33± 0.02
Ash Content	0.35±0.02	0.71±0.03	0.93±0.01
Fiber Content	0.18±0.01	0.67±0.01	0.10±0.002
Fat Content	0.67±0.04	0.13±0.03	0.03±0.001
Protein Content	0.83±0.05	0.08±0.003	0.33±0.04
Carbohydrate	0.79±0.02	0.14±0.04	0.39±0.02

Table 6: Proximate Composition Result of *Sassafras albidum, Detarium microcarpum* and *Echinaceae angustifolia* DC.

The results obtained revealed the presence of bioactive compounds in the crude methanolic extracts of these plants and hence showed a good correlation between the reported uses of these plants in traditional medicine against infectious diseases.

The presence of terpenoids, tannins, flavonoids, saponins, phlabotannin, and anthraquinone in the plants extracts justified its anti-parasitic, antibacterial, anti-inflammatory and anti-candidal properties. Alkaloids found in *Detarium microcarpum* and *Echinaceae angustifolia* confirmed the claim of the traditional healers that the plant can be used for malaria treatment [12].

The moisture content was high in *Sassafras albidum* of Lauraceae family and lower in *Detarium microcarpum* of Caesalpiniaceae family. The ash content was high in *Echinacea angustifolia* of Asteraceae family, but little amount was found in *Sassafras albidum* of Lauraceae family. The ash content followed the order 93.0% > 71.0% > 3.5% for *E. angustifolia, S. albidum* and *D. microcarpum* respectively. The fiber content was high in *Sassafras albidum* of Lauraceae family and lower in *Detarium microcarpum* of Caesapiniaceae family. The fiber content followed the order 67.0% > 10.0% > 8.0% for *Sassafras albidum, Detarium microcarpum and Echnaceae angustifolia* respectively. Crude fat was high in *Sassafras albidum* of Lauraceae family and lower in *Echnaceae angustifolia* of Asteraceae. The plants contained very little protein as indicated by the result of the analysis. However, the protein content is higher in *Detarium microcapum* and *Echinaceae angustifolia*. Carbohydrate content of *Sassafras albidum* and *Echinacea angustifolia* were lower than *Detarium microcarpum* which partly justified its use locally as food for its high content of carbohydrate. The Excel Microsoft office 2013 was used to analyze the data obtained from the cytotoxicity screening. The result indicated that all the three plants extracts were active in BST assay with LC50 of 63.1µg/ml, 158.49 µg/ml and 316.23µg/ml for *Sassafras albidum, Detarium microcarpum,* and *Echinaceae angustifolia DC* respectively and can be possible source of antitumor compounds.

Conclusion

This study availed us the medicinal potentials and nutrients value of the plants investigated. The Traditional Medicine Practitioners (TMP) have reasons for the use of these plants as remedy to ailments and as food. Currently a study is in an advanced stage to explore the antimalarial properties (in the *in vivo* and *in vitro*) of these plant species and to isolate pure compounds responsible for the observed bioactivity.

Recommendation

Similar studies and bioactivity screening are strongly recommended on these plants and other medicinal plants that grow in Kashere and environs.

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