Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Biology

Elixir Appl. Biology 45 (2012) 7652-7656

ISSN: 2229-712X

Evaluation of the chemical composition and antimicrobial activities of three Nigerian medicinal plants

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ARTICLE INFO

Article history:
Received: 10 February 2012;
Received in revised form:
17 March 2012;
Accepted: 28 March 2012;

Keywords
Acanthospermum hispidum,
Emilia coccinea,
Euphobia heterophylla,
Phytochemicals, Antibacterial,
Ethnomedicine.

ABSTRACT

Nigeran medicinal plants (*Acanthospermum hispidum* DC, *Emilia coccinea* (Sims) G. Don and *Euphobia heterophylla*) were analysed for their chemical composition. Phytochemical screening indicates the presence of saponins (0.22 to 0.37 mg/100g), flavonoids (0.96 to 1.87 mg/100g), alkaloids (1.52 to 1.71 mg/100g), phenols (1.07 to 1.55 mg/100g) and tannins (0.06 to 0.37 mg/100g). The medicinal plants contained ascorbic acid (24.35 to 31.79 mg/100g), riboflavin (0.04 to 0.31 mg/100g), thiamin (0.20 to 0.22 mg/100g) and niacin (0.08 to 0.10 mg/100g). These herbs are good sources of minerals such as Ca, P, K, Mg, Na, Fe and Zn. The plants gave appreciable antimicrobial activities *in vitro* against both gram-negative and gram-positive microorganisms with the zone of inhibition values ranging between (16.00mm – 32.00mm) and (11.00mm – 22.00mm) respectively. The importance of these chemical constituents is discussed with respect to the role of these herbs in ethnomedicine in Nigeria.

Introduction

In Nigeria, many indigenous plants are used in herbal medicine to cure diseases and heal injuries. Most of these higher plants are traditionally noted for their antimicrobial properties. Some of these have been biologically and phytochemically screened for their active and chemical constituents. Many of these metabolites have prominent effects on the animal system and microbial cells[1, 2]. Such medicinal plants include *Acanthospermum hispidum* (Asteraceae), *Emilia coccinea* (Asteraceae) and *Euphorbia heterophylla* (Euphorbiaceae). *A. hispidum* is an upright annual plant with dichotomous (Y-shaped) branching. The stem is densely covered with hairs. It is commonly found in cultivated upland crops, roadsides, pastures, waste areas and along railroads and cattle trails[3]. The extract of *A. hispidum* is used in the prophylaxis and therapy of retroviral disease in mammals and in the therapy of tumors[4]. It is also used in the treatment of jaundice and Herpes genitalis[3]. *E. coccinea* is an erect annual herb that is about 120 cm in height. It is a weed of roadsides, waste places and fallow land[4]. In Tanzania, eye inflammation is treated with aqueous extracts of *E. coccinea* and *Ipomea ericapa* as eye drops. Crushed green leaves of *E. coccinea* are used to treat wounds, sores and sinusitis. The roots are used to treat colic in babies in Tanzania and as a chest medicine in Kenya. It is also used to treat diarrhea[5]. *E. heterophylla* is a hardy, ruderal species, growing between 30 and 70 cm in height. The leaves at the upper end of the stalk are close to the cyathum and have a striking, scarlet red coloration. It is a perennial plant, with a round, slender, erect stem, 1 or 2 feet high, generally simple and smooth. *E. heterophylla* extract is used in the treatment of malarial and yellow fever. Its latex is also used as purgative[5].

The present study was designed to evaluate the mineral, vitamins, antimicrobial activities and secondary metabolite constituents of *A. hispidum*, *E. coccinea* and *E. heterophylla* commonly used in herbal medicine in Nigeria.

Materials and Methods

Collection of Plant Materials

All the medicinal plants were collected from Ado-Ekiti, Nigeria. The plants were examined, identified and authenticated at the Herbarium of the Department of plant Science and Forestry, Ekiti State University, Nigeria. The leaves were air-dried and milled with the aid of an electrical grinder and stored in airtight bottles before analysis.

Chemical Analysis

The major elements comprising Calcium, Phosphorous, Sodium, Potassium, Magnesium and trace elements (Iron and Zinc) were determined. The level of Lead and Cadmium were also determined according to the method[6].

About 2g of each of the plant samples were weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550^0C in a muffle furnace. The resultant ash was dissolved in 5ml of $HNO_3/HCl/H_2O$ (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionised water was added and heated until a colourness solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filter paper and the volume was made to the mark with deionised water. This solution was used for elemental analysis by atomic absorption spectrophotometer. Phosphorous content of the digest was determined colorimetrically according to the method[7].To 0.5 ml of the diluted digest, 4 ml of demineralised water, 3 ml of 0.75M H_2SO_4, 0.4 ml of 10% $(NH_4)_6MO_{24}.4H_2O$ and 0.4 ml of 2% (w/v) ascorbic acid were added and mixed. The solution was allowed to stand for 20min and absorbance readings were recorded at 660nm. The content of pbosphorous in the extract was determined. The minerals were reported in mg/100g.

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Preparation of fat free sample

2g of the sample were defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2h.

Alkaloid determination

5g of the sample were weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was filtered and the extract was concentrated using a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filteration and weighed[8, 9].

Tannin determination

500 mg of the sample was weighed into 100 ml plastic bottle, 50 ml of distilled water was added and shaken for 1h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made to the mark. Then 5 ml of the filtrate was pipette out into a tube and mixed with 3 ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120 nm wavelength, within 10 min. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured[10].

Determination of total phenol

For the extraction of the phenolic component, the fat free sample was boiled with 50 ml of ether for 15 ml of the extract was pipette into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made to mark and left to react for 30 min for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelengths[8, 9].

Saponin determination

20g of each plant samples were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4h with continuous stirring at about 55^0C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90^0C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of *n*-butanol was added. The combined *n*-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated in percentage[9].

Flavonoid determination

10g of the plant samples were extracted respectively with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed[11].

Determination of riboflavin

5g of the sample was extracted with 100 ml of 50% ethanol solution and shaken for 1h. This was filtered into a 100 ml flask. 10 ml of the extract was pipette into 50 ml volumetric flask. 10 ml of 55 potassium permanganate and 10 ml of 30% H_2O_2 were added and allowed to stand over a hot water bath for about 30 min. 2 ml of 40% sodium sulphate was added. This was made up to 50 ml mark and the absorbance measured at 510 nm in a spectrophotometer.

Determination of Thiamin

5g of the sample were homogenized with ethanilic sodium hydroxide (50 ml). It was filtered into a 100 ml flask. 10 ml of the filtrate was pipette and the colour developed by addition of 10 ml of potassium dichromate and read at 360 nm. A blank sample was prepared and the same wavelength.

Determination of Niacin

5g of the sample was treated with 50 ml of 1 N suphuric acid and shaken for 30 min. 3 drops of ammonia solution were added to the sample and filtered. 10 ml of the filtrate was pipette into a 50 ml volumetric flask and 5 ml potassium cyanide was added. This was acidified with 5 ml of 0.02 N H_2SO_4 and absorbance measured in the spectrophotometer at 470 nm wavelengths.

Determination of Ascorbic acid (vitamin C)

5g of each sample was weighed into an extraction tube and 100 ml of EDTA/TCA (2:1) extracting solution were mixed and the mixture shaken for 30 min. This was transferred into a centrifuge tube and centrifuged at 3000 rpm for about 20 min. It was transferred into a 100 ml volumetric flask and made up to 100 ml mark with the extracting solution. 20 ml of the extract was pipette into a volumetric flask and 1% starch indicator was added. These were added and titrated with 20% $CuSO_4$ solution to get a dark end point[12].

Determination of antibacterial activity

The antibacterial activities of the extracts were determined using the paper disc method. About 15 ml of molten nutrient agar was poured into the sterile Petri dishes and allowed to set. About 0.2 ml of a 24h old culture of each test organism was inoculated into the nutrient agar plate by sterile pipette. Sterile perforated filter paper discs were impregnated by soaking them in test tubes containing the extracts for 5 mins and allowed to dry before being placed on the nutrient agar plates were then incubated at 37^0C for 24h and the zone of inhibition were measured (to the nearest mm) with the aid of venial caliper.

Results and Discussion

Table 1 summarizes the quantitative determination of phytochemical constituents of *E. coccinea*, *A. hispidum* and *E. heterophylla*. High quantity of flavonoids, phenols and alkaloids were found on the three plant extracts. The flavonoid content was highest in *E. coccinea* (1.87 mg/100g) compared to *A. hispidum* and *E. heterophylla* which contain 1.33 mg/100g and 0.96 mg/100g respectively. The values of saponin and Tannin were very trace in the three plants. The mineral contents of the three plants are shown in Table 2. Potassium was the most abundant macro element present ranging from 12.23 mg/100g in *E. coccinea*, 11.44 mg/100g in *A. hispidium* to 10.34 mg/100g in *E. heterophylla*. This was followed by calcium, which was present from 6.85 mg/100g in *E. coccinea*, 4.02 mg/100g in *A. hispidum* to 4.71 mg/100g in *E. heterophylla*. Zinc was present at 1.24 mg/100g in *E.coccinea*, 0.91 mg/100g in *A. hispidum* and 0.75 mg/100g in *E. heterophylla*. Iron content was 2.51 mg/100g in *E. coccinea*, 4.40 mg/100g in *A. hispidum* and 2.90 mg/100g in *E. heterophylla*. Sodium content in the samples ranges from1.88 down to 1.64 mg/100g. The Magnesium values in the plant materials ranged between 2.02 and 1.86 mg/100g. The highest value was recorded in *E. heterophylla*.

Results of analysis of *E. coccinea*, *A. hispidum* and *E. heterophylla* in Table 3 showed that the plants are rich in vitamins most especially in ascorbic acid (vitamin C) which was found to be 31.79 mg/100g in *E. coccinea*, 28.72 mg/100g in *A. hispidum* and 24.35 mg/100g in *E. heterophylla*. Riboflavin,

thiamin and niacin were also detected in small quantities in the three plants. The result in Table 4 showed that all the three plants have antimicrobial activities. They all showed antimicrobial activities against both gram negative and gram positive microorganism. Though there were variations in their degree of antibacterial activity, the extracts were very potent against the test bacteria. The presence of phenolic compounds in the plants may have been responsible for the high antimicrobial activities of the plants. This agreed with the use of these plants for the treatment of typhoid fever, diarrhea and other bacterial infections particularly those caused by *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes* and *Salmonella typhi*. These findings supported the use of *E. coccinea* in treating the placenta and navel of newborn baby which not only heals in time but also prevent the formation of infections[13,].

The high saponin content of these plants justifies the use of the extracts from these plants especially E. coccinea to stop bleeding and in treating wounds. Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness[14, 15]. These properties bestow high medicinal activities on the extracts from *E. coccinea*, *A. hispidum* and *E. heterphylla*. Apart from saponins, other secondary metabolite constituents of *E. coccinea*, *A. hispidum* and *E. heterophylla* detected include alkaloids and flavonoids. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bacterial effects[16, 17]. They exhibit marked physiological activity when administered to animals. Flavonoids, on the other hand are potent water- soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity[15, 18, 19]. Flavonoids in intestinal tract lower the risk of heart disease. As antioxidants, flavonoids from these plants provide anti-inflammatory activity[16]. This may be the reason for the use of these plants for the treatment of wounds, sores and ulcers in herbal medicine. Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes. These perhaps, explain why traditional medicine healers often use these plants in treating wounds and sores [20].

These plants are good sources of ascorbic acid, riboflavin, thianin and niacin (Table 3). Natural ascorbic acid is vital for the body performance[15]. Lack of ascorbic acid impair the normal formation of intercellular substances throughout the body, including collagen, bone matrix and tooth dentine. A striking pathological change resulting from this defect is the weakening of the endothelial wall of the capillaries due to a reduction in the amount of intercellular substance[15, 21]. Therefore, the clinical manifestation of scurvy hemorrhage from mucous membrane of the mouth and gastro intestinal tract, anaemia, pains in the joints can be related to the association of ascorbic acid and normal connective tissue metabolism[15, 21]. This function of ascorbic acid also accounts for its requirement for normal wound healing. As a result of the availability of ascorbic acid in *E. coccinea*, *A. hispidum* and *E. heterophylla*, these plants are used in herbal medicine for the treatment of cold, colic in babies and as chest medicine[13, 15].

Riboflavin (vit. B_2), thiamin (vit. B_1) niacin (vit. B_3) are three of the eight B-complex vitamins including vitamins B_5, B_6, B_7, B_9 and B_{12}. These three vitamins work closely with each other to break the carbohydrates, fats, and proteins in food down into energy[15]. Deficiency symptoms of these vitamins can include; weakness and fatigue, eye disorders, chest pain, constipation, loss of appetite and weight loss, diarrhea, sore, swollen tongue or gum, depression etc[16]. The function of these three vitamins accounts for their requirement for normal body growth. Due to the presence of riboflavin, thiamin and niacin in *E. coccinea*, *A. hispidum* and *E. heterophylla*, these plants are used in ethnomedicine for the treatment of jaundice, eye inflammation, chest pain, and diarrhea and as a therapy for tumors.

Potassium was the most abundant macro element in the plants which was followed by calcium. Potassium and sodium are used for electrolyte balance in the body. This may be the reason why A. hispidum is used in the treatment of radiation disease. Normal extra cellular calcium concentrations are integrity intercellular cement substances[22]. Thus, the potentials of *E. coccinea* and *E. heterophylla* to stop bleeding and their uses in treating sores and wounds could be as a result of their high calcium content. Calcium and phosphorous are important in bone structure and growth. The low sodium content of *E. coccinea*, *A.hispidum* and *E. heterophylla* might be an added advantage due to the direct relationship of sodium intake with hypertension on human[23]. Magnesium is used by the body to help maintain muscles, nerves and bones. This function of magnesium may account for the usage of *A. hispidum* as an immunomodulator medicament[21]. The presence of zinc in the plants could mean that the plants can play valuable roles in the management of diabetes which result from insulin malfunction[22].

Conclusion

The knowledge of chemical, biological and therapeutic activities of medicinal plants used as folklore medicine is necessary. The minerals and phytochemical components of the medicinal plants may have been responsible for the antimicrobial activities of the plants[24, 25, 26]. This study therefore, has provided some biochemical basis for the ethnomedical use of the extracts from *E. coccinea*, *A. hispidum* and *E. heterophylla* in the treatment and prevention of infections. As rich sources of phytochemicals, minerals and vitamins, *E. coccinea*, *A. hispidum* and *E. heterophylla* can be potential sources of useful drugs.

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Table 1. Phytochemical composition of the leaves (mg/100g dry weight) of *Acanthospermum hispidum*, *Emilia coccicea* and *Euphobia heterophylla*

Phytochemicals	*E. coccinea*	*A.*	*hispidum*	*E. heterophylla*
Alkaloids	1.71	1.68		
Flavonoids	1.87	1.33		0.96
Phenols	1.55	1.12		1.07
Tannins	0.37	0.07		0.06
Saponins	0.37	0.33		0.22

Table 2. Mineral composition of the leaves (mg/100g dry weight) of *E. coccinea*, *A. hispidum* and *E. heterophylla*

Elements	*E. coccinea*	*A. hispidum*	*E. heterophylla*
Potassium	12.23	11.44	10.34
Sodium	1.88	1.70	1.64
Calcium	6.85	4.02	4.71
Magnesium	1.94	1.86	2.02
Manganese	0.63	1.10	0.83
Phosphorous	0.74	2.75	1.27
Iron	2.51	4.40	2.90
Zinc	1.24	0.91	0.75

Table 3. Vitamin composition (mg/100g dry weight) of *E. coccinea*, *A. hispidum* and *E. heterophylla*

Vitamin	E.coccinea	A. hispidum	E. heterophylla
Ascorbic acid	31.79	2872	24.35
Riboflavin	0.31	0.27	0.04
Thiamin	0.18	0.22	0.20
Niacin	0.10	0.09	0.08

Table 4. Antibacterial activities (mm) of the leaves extract of *E. coccinea*, *A. hispidum* and *E. heterophylla*

Test organisms	*E. coccinea*	*A. hispidum*	*E. heterophylla*
Klebsiella aerogenes	17.00	16.00	12.00
Enterococcus spp	17.00	18.00	11.00
Salmonella typhi	22.00	22.00	21.00
Shigella sonnei	15.00	24.00	16.00
Escherichia coli	32.00	26.00	24.00
Staphylococcus aureus	18.00	22.00	12.00
Pseudomonas aeruginosa	27.00	20.00	22.00