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COMPARATIVE ASSESSMENT OF TOTAL PHENOLIC CONTENT IN SELECTED MEDICINAL PLANTS

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Abstract

This study was to compare the total phenolic (TP) content in extracts from eleven plant materials collected at different geographical locations in Kenya, Nigeria, and USA. These plants have been selected because the majority of them are highly pigmented, from yellow to purple, and would therefore have economic value in industries for producing antioxidants and surfactants. Two of them were collected from the industrial and domestic waste outlets. Each analysis was achieved using the Folin-Ciocalteu technique. The order of decreasing phenolic acid content as gallic acid concentration (mg/g dry weight) was *Prunus africana* (55.14) > *Acacia tortilis* (42.11) > *Khaya grandifoliola* (17.54) > *Curcuma longa* (17.23) > *Vernonia amygdalina* (14.9) > *Russelia equisetiformis* (14.03) > *Calendula officinalis* (7.96) > *Phragmites australis* (control) (7.09) > *Rauwolfia vomitoria* (6.69) > *Phragmites australis* (industrial) (6.21) > *Cnidioscolus aconitifolius* (5.6). The TP contents of *Spartina alterniflora* species were below the detection limit.

Keywords

Medicinal plants; phytochemicals; total phenolic content; Folin-Ciocalteu

INTRODUCTION

Phenols are important constituents present in most plant materials. They encompass a vast number of groups including flavanoids, phenolic acids, stilbenes, lignans, lignins and tannins [1]. Tannins have the highest molecular weight of the phenolic acids and, aside from medicinal value, are known for their astringency and usefulness in the leather industry [2]. Secondary metabolites of phenol nature may act as part of the plant's defense against insects, animals or pathogens [2]. In humans, they have various properties including anti-bacterial, anti-inflammatory, anti-helminthic and anti-septic [2]. However, at high doses, beyond levels found in plants, these same metabolites may be toxic [3]. One important role of phenolics is their antioxidant activity. Antioxidants have won acclaim for their touted health benefits as cardiovascular protectants, anti-aging effects and possible anti-cancer activity [4,5]. Plants have varying levels of total phenolic (TP) content. These plants have potential of being antioxidant sources and arrest free radical damage. The aim of this study was therefore, to determine the TP content in medicinal plants collected at various locations in Nigeria, Kenya and Maryland (USA). The plants were selected based on the availability of plant materials and because of the many significant studies on their medicinal uses, such as anti-inflammatory, anti-diabetic, anti-hypertensive, anti-cancer and anti-viral properties [6,7,8,9,10,]; and removal of pollutants from wetlands [11,12]. The plants analyzed were: *Russelia equisetiformis* (*RE*) (Scrophulariaceae), *Prunus Africana* (*PA*) (Rosaceae), *Cnidoscopus aconitifolius* (*CA*) (Euphorbiaceae), *Acacia tortilis* (*AT*) (Fabaceae), *Curcuma longa* (*CL*) (Zingiberaceae), *Rauwolfia vomitoria* (*RV*) (Apocynaceae), *Calendula officinalis* (*CO*) (Asteraceae), *Khaya grandifoliola* (*KG*) (Meliaceae), *Vernonia amygdalina* (*VA*) (Asteraceae), *Spartina alterniflora* (*SA*) (Poaceae) and *Phragmites australis* (*PAU*) (Poaceae).

These plants have rich histories of medicinal value. Research demonstrates the anti-diabetic activity of *CA* [6,23,29] and anti-inflammatory activity of *RE* [8]. *CO* has a history of use for its' anti-inflammatory and anti-cancer properties [13]. *RV* is known for its' anti-inflammatory and anti-arthritic actions [9]. *CL* has an extensive history as an anti-inflammatory as well as an anti-cancer agent [10]. *KG* has exhibited some anti-malarial activity [14]. *PA* plays a significant role in reducing inflammation and other symptoms associated with benign prostatic hyperplasia (BPH) [15]. *VA* has been reported to possess both anti-malarial and anticancer activity [16,17]. It has also been used as a treatment for sexually transmitted diseases [18].

The *SA* and *PAU* are invasive plants and environmental pollutant removers [12]. In this study they were growing wildly in Edgewater (control) and Fort McHenry River (industrial outlet), all from part of the Chesapeake Bay river, Maryland, USA. Two samples of each *SA* and *PAU* were analyzed, respectively. One sample was taken from an industrial polluted area in Fort McHenry; the other sample was collected from Edgewater (control) where there was no industrial pollution. Their TP contents were to be analyzed for comparison. The Folin-Ciocalteu reagent, which has been used extensively as a complexing coloring reagent [19–22], was used for measuring the TP contents.

DISCUSSION

The results of this analysis are shown in Table 1. There are three (3) significant findings of this study. First, the order of decreasing TP content as gallic acid equivalent (mg/g dry weight) was *Prunus africana* (55.14) > *Acacia tortilis* (42.11) > *Khaya grandifoliola* (17.54) > *Curcuma longa* (17.23) > *Vernonia amygdalina* (14.9) > *Russelia equisetiformis* (14.03) > *Calendula officinalis* (7.96) > *Phragmites australis* (control) (7.09) > *Rauwolfia vomitoria* (6.69) > *Phragmites australis* (industrial) (6.21) > *Cnidoscopus aconitifolius* (5.6). Secondly, the TP contents of *Spartina alterniflora* species were below the detection limit of 0.01 Absorbance.

Lastly, the ranges of coefficient of variations for the intra- and inter-assays for the absorbance of various filtered extracts of plant materials were 5.8–8.6 and 9.8–11.4, respectively.

The TP content of some of the plants in this study have been reported elsewhere. Kahkonen et al. [1] reported the TP content of *PAU* to be 6.2 mg/g dry wt. GAE and 1.2 mg/g dry wt. GAE for the leaf and stalk, respectively. This is comparable to the results found in this study. The TP content of *CA* was reported to be 2.91 ± 0.019 mg/g of chlorogenic acid equivalents of fresh weight for the raw sample and 2.24 ± 0.038 mg/g for cooked sample. The Oxygen radical absorbance capacity (ORAC) value, which is a measurement of antioxidant capacity, was 15.3 [23]. In the present studies, the value obtained (5.6 ± 0.75 mg/g dry weight GAE) for *CA* was quite high. The difference may be due to different geographical collection areas. The *CA* we used was harvested from the western part of Nigeria [6] while the referenced paper above was collected from the boarder of USA.

Various studies demonstrate that the plants with the highest TP content have potential for greater antioxidant activity [24–28]. In addition Cai et al. found that the antioxidant activity not only was associated with the TP content but also with the molecular structure, primarily the number and position of the primary hydroxyl group [25]. Previous anti-radical and antioxidant activity has been reported for some of the plants. Curcuminoids, found in *CL* are phenolics found in the Zingiberaceae family. These constituents demonstrated significant anti-radical activity [25]. Also, *VA* has demonstrated antioxidant activity against acetaminophen-induced hepatotoxicity [27]. Future studies will quantify and compare the ORAC with TP content of the plants to assess the correlation between the TP content and antioxidant activity.

EXPERIMENTAL

Materials and Methods

The Folin-Ciocalteu was prepared as described by Singleton and Rossi [21]. Gallic acid was obtained from Sigma Aldrich. Sodium carbonate was purchased from Fisher Scientific and a 20% solution prepared from it.

Collection of Plant Materials

CA leaf, *VA* leaf, *RE* whole plant and *KG* bark were collected from southwestern Nigeria during the rainy season, and identified using descriptions in literature [22]. Authentication was done in the Department of Pharmacognosy, University of Ibadan, and voucher number obtained from the Nigeria Forestry Research Institute, Ibadan, Nigeria. The plant material was later carefully air-dried indoors at room temperature for three days before drying in an oven between 40–50°C; and comminuted into moderately coarse powder and kept in sealed polythene bags until chemical analysis. *PA* and *AT* bark were randomly collected mainly from the Government Forest Reserve in the Taita Hills and from other various sites along the roads leading towards this forest reserve; KEMRI Botanical Garden at Taveta and other various sites along the road leading towards this botanical garden; KEMRI Botanical Garden and Government Forest Reserve at Kwale and various other sites along the road to Kinango; KEMRI Botanical Garden at Mbagathi plus various other sites within and around Nairobi. The samples were collected more than once from all these sites during both wet and dry seasons.

In the United States of America, *CL* root powder and *CO* dried flowers were obtained from Pacific Botanicals, Grants Pass, Oregon 97527 while the *RV* root was obtained from Gaia Herbs, Brevard, North Carolina 28712. The *SA* and *PAU* samples were collected from the Smithsonian Environmental Research Center, Edgewater (control) and Fort McHenry River (industrial outlet), part of the Chesapeake Bay river, Maryland, USA during the spring season.

Extraction

5.0 g of each of plant materials was added to 40 mL of a mixture of ethanol/acetone/water/acetic acid (40:40:20:0.1) solution [23]. The resultant mixtures together with the plant material were allowed to stand in a 60 °C water bath for 1 hour. After cooling for 15 minutes, the extracts were filtered using a 0.45µm Sterile Millex-HA filter, Millipore, Bedford, MASS. 01730.

Determination of phenolic content

By serial dilutions, various concentrations of 0.15 mg/mL, 0.45 mg/mL, 0.75 mg/mL, 1.05 mg/mL and 1.50 mg/mL gallic acid were prepared from its stock solution of 5 mg/10 mL. The absorbance of each was measured several times with a suitable BioSpec-1601 spectrometer from Shimadzu Scientific Instruments, Inc. Columbia, Maryland, USA, and the average values plotted against concentrations to prepare the calibration curve. The phenolic content of the filtered extracts, as gallic acid equivalent were determined from this curve. 1.0 mL of the filtered extract in a 1:100 dilution was added to 6.0 mL of double-distilled de-ionized water, followed by 0.5 mL of the Folin-Ciocalteu reagent. After 30 seconds but before 8 min, 1.5 mL of Na₂CO₃ solution was added. Finally, 1.0 mL of double-distilled deionized water was added to give a total volume of 10 mL. After 2 hours, the absorbance of the solutions was read with the spectrometer at a wavelength of 610nm. A blank solution was prepared and read similarly. Series of triplicate measurements were taken and averaged. From the calibration curve, the mean absorbance was used to determine the total phenolic content of the filtered extract equivalent to gallic acid (R²=0.983). The intra- and inter-assays and calculation of coefficient of variations for the different period of measurements were determined.

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Table 1

Total Phenolic Content in Plants

Plant	Phenol content in *GAE (mg/g dry weight)
PA	55.14±1.5
AT	42.11±2.4
KG	17.54±0.65
CL	17.23±0.35
VA	14.9±0.2
RE	14.03±0.9
CO	7.96±0.85
PAU (Control)	7.09±1.6
RV	6.69±0.5
PAU (Industrial)	6.21±1.6
CA	5.6±0.75
SA(Control)	BDL
SA(Industrial)	BDL

* GAE = Gallic acid equivalent (Dilution factor corrected) Absorbance wavelength: 610nm Below Detection Limit (BDL)