**World Journal of Pharmaceutical Sciences**
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers 
Available online at: http://www.wjpsonline.org/
**Original Article**

# Chemical study, antiradical and antibacterial potential of the extracts of *Ximenia americana* and *Cussonia arborea* of Benin

Koudoro Yaya Alain, Mahudro Yovo, Yehouenou Boniface, Agbangnan Dossa Cokou Pascal, Tchobo Fidèle Paul, Alitonou Guy Alain, Sohounhloue C. K. Dominique*

Université d'Abomey-Calavi (UAC), Ecole Polytechnique d'Abomey-Calavi (EPAC), Laboratoire d'Etude et de Recherche en Chimie Appliquée (LERCA), 01 BP 2009 Cotonou République du Bénin

**Received: 26-10-2014 / Revised: 09-11-2014 / Accepted: 24-11-2014**

## ABSTRACT

This paper reports the chemical and biological studies of two plants, *Ximenia americana* and *C. arborea* used by farmers in Benin in the treatment of animal's gastrointestinal diseases. We noted in both samples, the presence of several secondary metabolites such as saponins, catechin tannins, mucilages, flavonoids, anthocyanins, reducing compounds, sterols and terpens. The total polyphenol content was higher in the aqueous extract of two plants than in ethanolic and hydroethanolic extracts. Those of aqueous extracts were respectively (6.419 ± 0.335) mg EA / g MS for *X. americana* trunk bark and (3.110±0.132) mg EA /g DM for *C. arborea*. The trunk bark extracts of *X.americana* have DPPH scavenging activities of 5µg / ml and 4µg / ml, betters than those of *C. arborea*. The ethanolic extract of this plant (*X. americana*) was more active ($IC_{50}$=4µg/ml) than BHA ($IC_{50}$= 4.8µg / ml) which was a synthetic antiradical. The results of antibacterial activity indicate that all extracts (ethanolic, hydroethanolic and aqueous) of the trunk bark of *X. americana* have inhibited strains of *S. aureus*, *K. pneumoniae* and *S. typhi*. The three extracts of this plant have showed a bactericidal activity against *E. coli* and aqueous extract also displayed a bactericidal one against *K. pneumonia. S. typhi* got a pronounced sensitivity with ethanolic and aqueous extracts of trunk bark of *C. arborea.*

**Keyword**s: *Ximenia americana*, *Cussonia arborea*, secondary metabolites, antiradical, bactericidal

## INTRODUCTION

Men have always delved from the nature what to feed, to wear and to heal [1]. Herbal medicines were the most widely used way to solve the problems of human and animal health from the beginning of human kind. *Cussonia arborea* Hochst (Araliaceae) is a tree from 7 to 10 m height, usually glabrous, rarely tomentose and characterized by stalked leaves, simple, palmate or lobed. Its flowers are greenish and oval fruits are often urceolair [2]. *Ximenia americana* (Olacaceae) is a shrub or small tree up to 6 m height. Its leaves are alternate, elliptic, thin, or clustered on straight shoots, tapered and base rounded [2].

Both plants belong to the therapeutic arsenal used by farmers to treat some animal's diseases, such as gastrointestinal diseases, diarrhea, internal parasites, foot and mouth diseases... etc [3-5]. Very few works exist in the literature related to the chemical and biological study of *C. arborea* and *X. americana*. In Benin, these plants commonly used by traditional healers and ranchers for their curative properties in the treatment of several pathologies, have not been the interest of scientific investigations. It was therefore appropriate to focus thinking about the chemical and biological studies of extracts of these two plants extracts. This study aims to identify secondary metabolites in plants, quantify polyphenolic compounds and evaluate the antibacterial and antiradical activities of ethanolic, hydroethanolic and aqueous extracts of these plants.

## MATERIAL AND METHODS

**Plant material:** The plant material used in this study was made from the trunk bark of *Ximenia americana* and *Cussonia arborea* harvested respectively in Bembèrèkè and Nikki, northern areas of Benin.

**Animal material:** It consists of the reference strains of *Staphylococcus aureus* (ATCC 27844),

***Corresponding Author Address:** SOHOUNHLOUE C. K. Dominique, Université d'Abomey-Calavi Laboratoire d'Etude et de Recherche en Chimie Appliquée (LERCA), 01 BP 2009 Cotonou République du Bénin, E-mail : csohoun@gmail.com*

*Escherichia coli* (O: 157H7), *Salmonella typhi* (R.0951401) and *Klebsiella pneumoniae* (ATCC 35657). These strains were provided by the National Health Laboratory of Benin.

**Methods:** After collecting over the plant material, the samples were dried at laboratory temperature (25°C-30 °C) until their stabilization and then reduced in powder.

**Identification of secondary metabolites:** Determination of secondary metabolites was made by staining reactions and precipitation specific to each metabolite family.

**Flavonoids**: Flavonoids identification was carried out by the test of cyanidin [6].

**Tannins**: They have been highlighted by the Stiasny test [7].

**Saponins**: The saponins were determined by foam test; degree of aqueous decoction dilution giving a persistent foam after shaking [8], [9]

**Polyphenols**: Identification of compounds belonging to the group of polyphenols was made by the reaction with ferric chloride [8].

**Terpenes and sterols**: Sterols and terpens have been identified by the Liebermann-Burchard test [10].

**Alkaloids**: Alkaloids were identified by Meyer test and confirmed by Bouchardat test [11].

**Anthraquinone:** They were identified by Bornträger test [9].

**Mucilages**: Obtaining a decoction of a flocculent precipitate in ethyl ether indicated the presence of mucilages [12].

**Coumarins**: Coumarins were identified by UV fluorescence at 365 nm [7].

**Volatile compounds**: The volatile compounds were identified by the hydro distillation method using an extractor of Clevenger type [13-15].

**Preparation of extracts:** The technique used was that of maceration. 50g of each powder sample were introduced into a 500 ml flask containing 250 ml of extraction solvent (ethanol, water or ethanol-water 50/50). The flask was stoppered and stirred continuously for 72 hours. After filtration, the extracts were evaporated to dryness at 40 °C using a rotary evaporator Heidolph kind. The yield (Y) of extraction was calculated by the formula below

Y (%)=(Mass of extract)/ (Mass of plant material used) X100

**Determination of polyphenolic compounds**

**Total polyphenols**: The total phenolic content of the various extracts was quantified using the Folin–Ciocalteu reagent according to Singleton *et al*. [16-17]. This method consists to use a mixture of phosphotungstic and phosphomolybdic acids which was reduced during the oxidation of phenols into a mixture of tungsten blue oxide and molybdenum [18]. The absorbance was measured by a spectrophotometer (JENWAY 50/60 Hz) to 765 nm. Gallic acid was used as reference and the total polyphenol content in the extract was expressed by mg of Gallic acid equivalent per gram of dry matter.

**Total Flavonoids**: The method of aluminum trichloride ($AlCl_3$) was used to quantify the total flavonoids. This technique was based on the formation of the aluminum complex flavonoids that had a maximum absorption at 500 nm [19-20].

**Condensed tannins**: The condensed tannins dosing was achieved by the method of sulfuric vanillin [21, 22]. The principle of this assay was based on the binding of vanillin aldehyd group on the carbon in position 6 of the ring of the catechol to form a red colored complex chromophore which absorbed at 510 nm.

**Evaluation of scavenging activity:** The scavenging activity was evaluated by the DPPH method. The principle of this method was based on measuring the trapping free radicals in a solution of DPPH. This trapping was indicated by the disappearance of the purple color of DPPH. The mixture of DPPH solution and the sample was left in the darkness for an hour and the absorbance measured at 517 nm [23, 24]. The trapping percentage was determined by the formula: $P= ((Ab_W-Ab_S)/Ab)X100$; P: percentage of trapping; $Ab_W$: absorbance of the white; $Ab_S$: Absorbance of the sample

**Determination of antibacterial activity:** The antibacterial activity was evaluated in microplates and in Petri dishes according to literature [25-27].

## RESULTS AND DISCUSSION

The secondary metabolites identified in the trunk bark of *C. arborea* and *X. americana* were shown in Table 1. Various secondary metabolites have been highlighted in the trunk bark of both plants. *C. arborea* was rich in polyphenols, anthocyanins, proteins, flavonoids, tannins, reducing compounds, sterols and terpens. Salihu and Ado [28] identified in the trunk bark of this plant harvested in Kaduna (Nigeria) the alkaloids which were absent in the sample from Benin. In the trunk bark of *X. americana*, we noted the presence of saponosides, catechic tannins, gallic tannins, polyphenols, flavonoids, alkaloids, leuco anthocyanins, reducing compounds, quinones, mucilages, proteins, sterols and terpenes whereas Maikai *et al*. [29] highlighted the presence of tannins, saponosids, flavonoids, cardiotonic compounds and quinones in the sample collected in Zaria (Nigeria). According to the work

of Maikai *et al*.[30] the species of Nigeria was rich in alkaloids, anthraquinones, flavonoids, tannins, cardiac glycosides, sterols and terpenes. The variation of our results compared to previous work might be related to the time of harvest, the nature of soil or climatic factors [31-32]. The diversity of the secondary metabolites in the trunk bark of these plants could explain their use in the treatment of inflammatory; diarrhea; parasitic, infections and gastrointestinal diseases in livestock. The presence, for example, of tannins and flavonoids in the trunk bark of these two plants, justified their use in the treatment of diarrhea and gastro-intestinal animals diseases [33, 34].

**Extraction yields:** The extraction yields of the trunk bark of *C. arborea* and *X. americana* showed in Table 2. In this table, yields of ethanolic, hydroethanolic and aqueous extracts of *C. arborea* trunk bark varied from 18.8% to 41.6% with high yield in the ethanolic extract while those of trunk bark extracts of *X. americana* vary from 14.8% to 24%.

**Quantification of phenolic compounds**

***Total polyphenol content:*** The total polyphenols content expressed in mg of Gallic acid equivalent per gram of dry matter extracted from the trunk bark of *C. arborea* and *X. americana* was indicated by the figure 1. The total polyphenols contents of ethanolic, hydroethanolic and aqueous extracts of stem bark of *C. arborea* were respectively (0.782±0.074), (1.271± 0.167) and (3.110 ± 0.132) mg GAE/g DM while those of *X. americana* were (2.805± 0.115), (3.344 ± 0.234) and (6.419±0.335) mg GAE/g DM. The highest levels of total polyphenols were obtained in the aqueous extract of the two plants with the highest value in the trunk bark of *X. americana*.

It follows from those results that the polarity increasing of the extraction solvent promotes the extraction of total polyphenols in the stem barks of the two plants.

***Total flavonoids content:*** The Figure 2 showed the content of total flavonoids extracted from the stem bark of *C. arborea* and *X. americana* expressed in mg catechin equivalent per gram of dry matter. The total flavonoid content of the ethanolic extract of trunk bark of *C. arborea* was (7.455 ± 0.628) mg CE/g DM while those of the ethanolic and aqueous extract were respectively (5.246±0.211) and (9.499±0.685) mg CE/g DM. The total flavonoids content of trunk bark extracts of *X. Americana* were (60.226 ± 0.921), (43.747 ± 1.279) and (59.499 ± 0.023) mg CE/g DM respectively for ethanolic, hydroethanolic and aqueous extracts. We noted through those results that the content of total flavonoids was greater in extracts from *X. americana* than that of *C. arborea*.

***Condensed tannins content:*** Figure 3 displayed the content of condensed tannins extracts from the trunk bark of *X. americana* expressed in mg catechin equivalent per gram of dry matter. The levels of condensed tannins of ethanol, hydroethanolic and aqueous extracts were respectively (139.395±0.921) mg CE/g DM (143.814±3.289) mg CE/DM and g (90.046 ± 0.263) mg CE/g DM for trunk bark of *X. americana*. The highest level was obtained in the hydroethanolic extract of this plant.

**Radical scavenging activity of extracts from the trunk barks of *X. americana* and *C. arborea:*** For all three extracts from the trunk bark of *X. americana* we noted a sudden increase trapping rate at low concentrations, which becoming almost virtually constant at 100% at high concentrations (Figure 4). From these curves, the $IC_{50}$ (concentration of the extract to 50% of trapping free radicals) of the ethanolic, hydroethanolic and aqueous extracts of trunk bark of *X. americana* determined by graphical extrapolation were respectively of 5µg/ml, 4µg/ml and 5µg/ml. Regarding the extracts of trunk bark of *C. arborea*, the concentrations treated showed a gradual increase of the percentage of free radicals trapped. These curves were used to determine the concentrations of the extracts which scavenged 50% of free radicals ($IC_{50}$). These concentrations were respectively 100µg/ml; 80µg/ml and 70µg/ml for ethanolic, hydroethanolic and aqueous extracts. All extracts from the trunk bark of *X. americana* showed more interesting scavenging activity than those of *C. arborea*. For *X. americana*, the hydroethanolic extract showed the most interesting antiradical activity (more pronounced than that of the BHA a synthetic antiradical) whereas the aqueous extract of *C. arborea* was the more active but less active than BHA. The extracts from the trunk bark of *X. americana*, which already was proved richer in polyphenols than those of the trunk bark of *C. arborea*, have showed the more pronounced free radical scavenging activity. Those activities of extracts from this plant could be attributed to their higher content of phenolic compounds as mentioned by several studies in the literature on plant extracts [24, 35, 36].

**Antibacterial activity of extracts**

***C. arborea* trunk bark:** The minimal inhibitory concentrations (MIC), Minimal Bactericidal Concentrations (MBC) and antibiotic power of the extracts from the trunk bark of *C. arborea* were shown in table 3. The aqueous and ethanolic extracts of *C. arborea* have bactericidal concentration of 100 mg/ml and an interesting

antibiotic activity power (MBC/MIC=2) with the strain of *S. typhi*. In the contrary, the hydroethanolic extract had showed only an inhibitory activity with this strain. The three extracts from the trunk bark of *C. arborea* (ethanolic, hydroethanolic and aqueous) have inhibited *S. aureus*. In contrary, the ethanolic extract showed bacteriostatic activity with the strain of *K. pneumoniae*. The ethanolic extract of trunk bark of *C. arborea* showed an interesting antibacterial activity with *E. coli*, while the aqueous extract showed a bacteriostatic effect.

***X. americana* trunk bark:** Minimal Inhibitory Concentration (MIC), Minimal Bactericidal Concentration (MBC) of the extracts from the trunk bark of *X. americana* as well as the antibiotic power of these extracts were consigned in Table 4. From reading this table, we noted that the different extracts (ethanol, hydroethanolic and aqueous) hold relative antibacterial activities against the bacterial strains. The MIC of this plant extracts varied from 0.39 mg / ml to 100 mg / ml with the four strains tested. The hydroethanolic extract has inhibited *S. aureus* (MIC=0.39 mg / ml) and *S. typhi* strain (MIC=3.12 mg/ml*). K. pneumoniae* was more sensitive to the ethanolic extract of *X. americana* (0.39 mg / ml), while the strain of *E. coli* was sensitive to ethanolic and hydroethanolic extracts of this plant. Overall, the hydroethanolic extract was the most active against the strains of *E. coli* and *S. typhi*. The aqueous extracts of *X. americana* were less active on the strains tested. The ethanolic, hydroethanolic and aqueous extracts of the trunk bark of *X. americana* were the only ones that possessed a bactericidal respectively against *E. coli* and *K. pneumoniae*. The antibacterial activities noted with extracts from trunk bark of *C. arborea* and *X. americana* could probably be related to the chemical profile of the stem bark of these plants either to the action of a secondary metabolites or to a synergy effect between secondary metabolites (polyphenols, tannins, saponins, flavonoids, alkaloids) [37,38].

## CONCLUSION

On the basis of the results of present study we noted in both samples, the presence of several secondary metabolites. The total polyphenol content was higher in the aqueous extract of the two plants than ethanolic and hydroethanolic extracts. The trunk bark extracts of *X. americana* showed better antiradical activity compared with those from *C. arborea* trunk bark. The hydroethanolic extract of the trunk bark of *X. americana* was the most active ($IC_{50}$=4µg/ml) than BHA ($IC_{50}$=4.8µg/ml), a synthetic antiradical. The results of antibacterial activities showed that all extracts (ethanolic, hydroethanolic and aqueous) of *X. americana* had inhibited strains of *S. aureus*, *S. typhi* and *K. pneumoniae*. These extracts showed bactericidal activity against *E. coli* strain while only the aqueous extract of this plant showed bactericidal activity against *K. pneumoniae*. *S. typhi* showed a very pronounced sensitivity with the aqueous and ethanolic extracts of the trunk bark of *C. arborea*. The diversity of secondary metabolites and biological activities noted in the trunk bark of *C. arborea* and *X. americana* could justify the use of these two plants by farmers to treat livestock diseases. It is therefore appropriate to guide future studies towards the isolation and characterization of bioactive compounds present in the extracts from the trunk bark of *X. americana*.

## ACKNOWLEDGMENTS

The authors of this work thank sincerely the rectoral team of University of Abomey-Calavi for his financial support through the project “PHARMAVET".

**Table1. Metabolites identified in the trunk bark of *C. arborea* and *X. Americana***

| Secondary metabolites | | *C. arborea* | *X. americana* |
|---|---|---|---|
| **Alkaloids** | | - | + |
| **Polyphenols** | | + | + |
| **Flavonoids** | | + | + |
| **Anthocyanins** | | - | + |
| **Leuco-anthocyanins** | | + | + |
| **Anthraquinones** | | - | - |
| **Free anthraquinones** | | - | - |
| **Combined anthraquinones** | O-heterosides | - | - |
| | O-heteroside with reduced genine | - | - |
| | C-heterosides | - | - |
| **Reducing Compounds** | | + | + |
| **Tannins** | Gallic | - | + |
| | Catechic | + | + |
| **Sterols and terpenes** | | + | + |
| **Mucilages** | | + | + |
| **Saponosides** | | + | + |
| **Coumarines** | | - | - |
| **Quinones** | | - | + |
| **Proteins** | | + | + |
| **Essential oil** | | - | - |

+: presence;-: Absence

***Table2. Extraction yields of C. arborea and X. Americana trunk bark***

| | Yieds(%) | |
|---|---|---|
| Extracts | *C. arborea* | *X. americana* |
| Ethanolic | 18.80 | 14.80 |
| hydroethanolic | 41.60 | 18.80 |
| Aqueous | 21.20 | 24.00 |

**Table 3. Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of *C. arborea* extracts.**

| Microorganism strains | Extracts | Concentrations (mg/ml) | | |
|---|---|---|---|---|
| | | *C. arborea* | | |
| | | MIC | MBC | MBC/MIC |
| *S. typhi* | Ethanolic | 50.00 | 100.00 | 2.00 |
| | hydroethanolic | 6.25 | >100.00 | >16.00 |
| | Aqueous | 50.00 | 100.00 | 2.00 |
| *S. aureus* | Ethanolic | 100.00 | >100.00 | >1.00 |
| | hydroethanolic | 50.00 | >100.00 | > 2.00 |
| | Aqueous | 2.50 | >100.00 | > 40.00 |
| | Ethanolic | >100.00 | >100.00 | >1.00 |
| *E. coli* | hydroethanolic | 25.00 | 50.00 | 2.00 |
| | Aqueous | 6.25 | 100.00 | 16.00 |
| *K. pneumoniae* | Ethanolic | 12.50 | 100.00 | 8.00 |
| | hydroethanolic | >100.00 | >100.00 | >1.00 |
| | Aqueous | >100.00 | >100.00 | >1.00 |

**Table 4. Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of *X. americana* extracts.**

| Microorganism strains | Extracts | Concentrations (mg/ml) | | |
|---|---|---|---|---|
| | | *X. americana* | | |
| | | MIC | MBC | MBC/MIC |
| *S. typhi* | Ethanolic | 12.50 | >100.00 | > 8.00 |
| | hydroethanolic | 3.12 | >100.00 | > 32.00 |
| | Aqueous | 25.00 | >100.00 | > 4.00 |
| *S. aureus* | Ethanolic | 100.00 | >100.00 | > 1.00 |
| | hydroethanolic | 0.39 | >100.00 | > 256.00 |
| | Aqueous | 3.13 | >100.00 | > 32.00 |
| *E. coli* | Ethanolic | 0.78 | 3.12 | 4.00 |
| | hydroethanolic | 0.78 | 1.56 | 2.00 |
| | Aqueous | 50.00 | >100.00 | > 2.00 |
| *K. pneumoniae* | Ethanolic | 0.39 | >100.00 | >256.00 |
| | hydroethanolic | 1.56 | >100.00 | > 64.00 |
| | Aqueous | 6.25 | 12.50 | 2.00 |

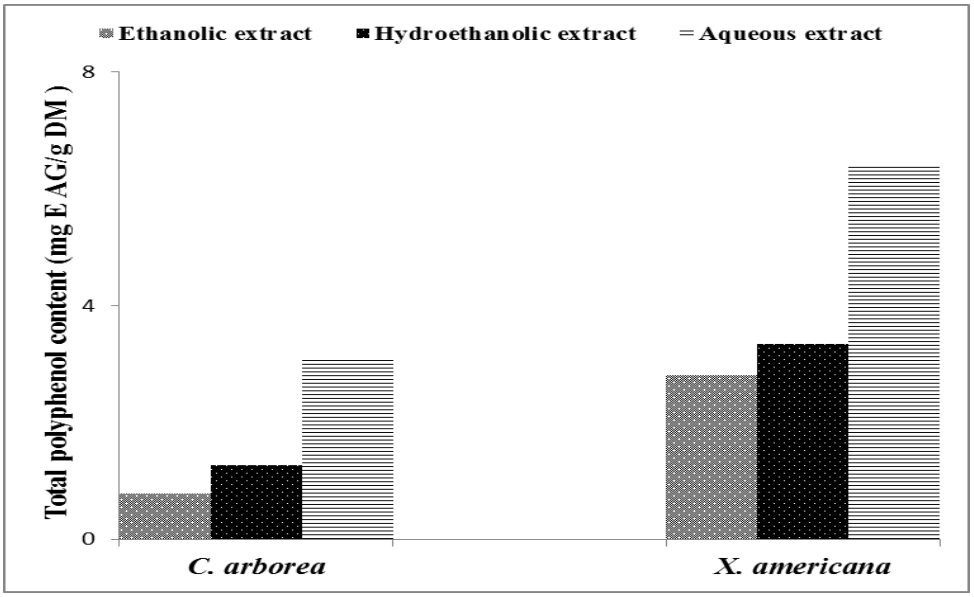



***Figure1*. Content of polyphenols in ethanolic, hydroethanolic and aqueous extracts of the trunk bark of *C. arborea* and *X. Americana***

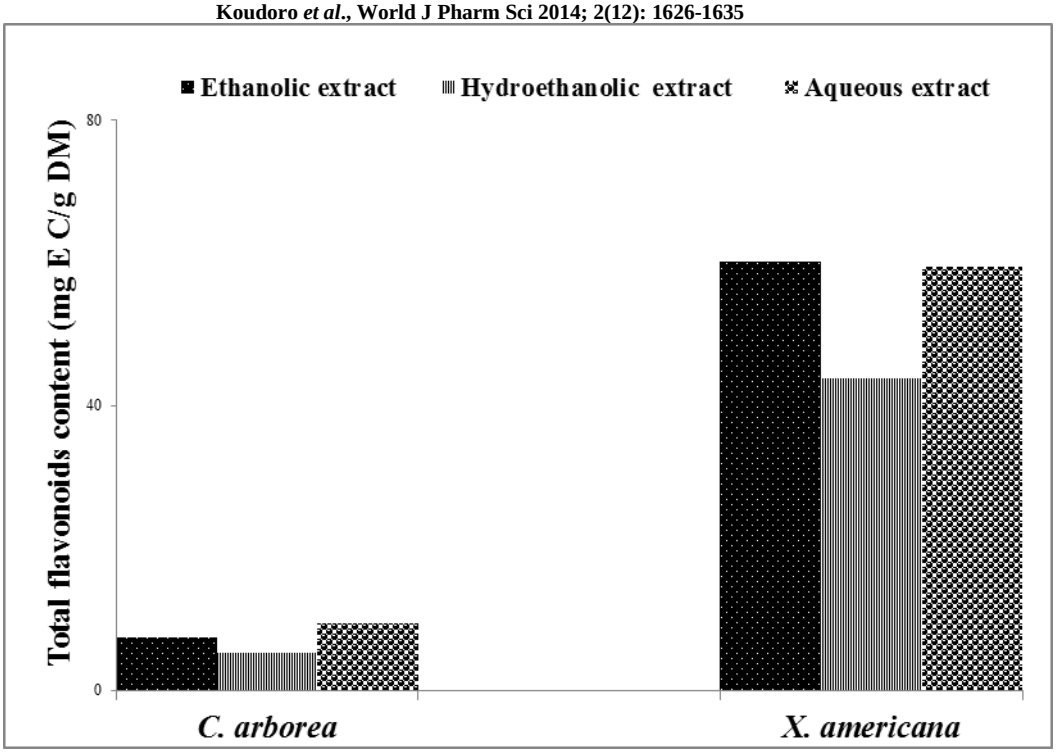



***Figure 2*. Total flavonoids content of ethanolic, hydroethanolic and aqueous extracts of trunk bark of *C. arborea* and *X. americana.***

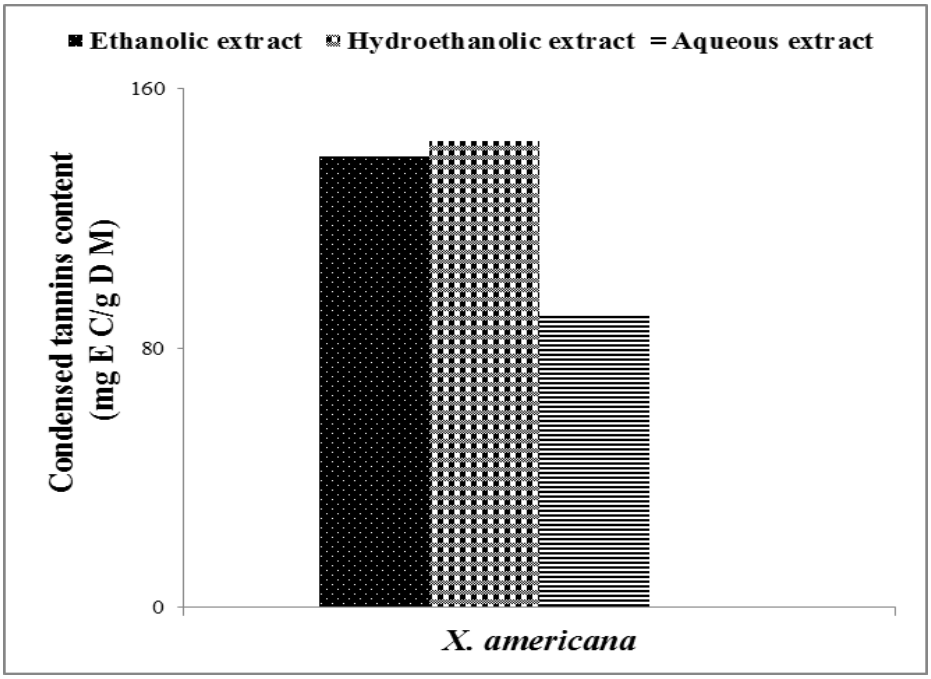
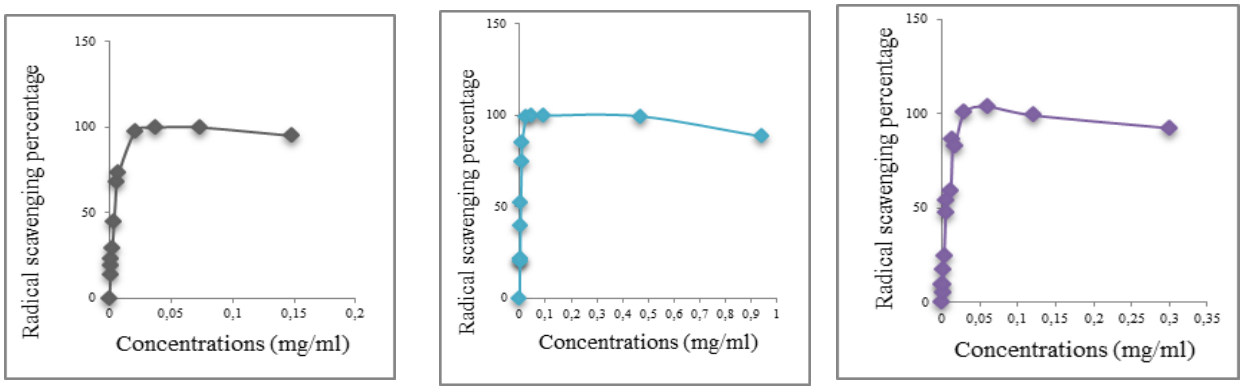
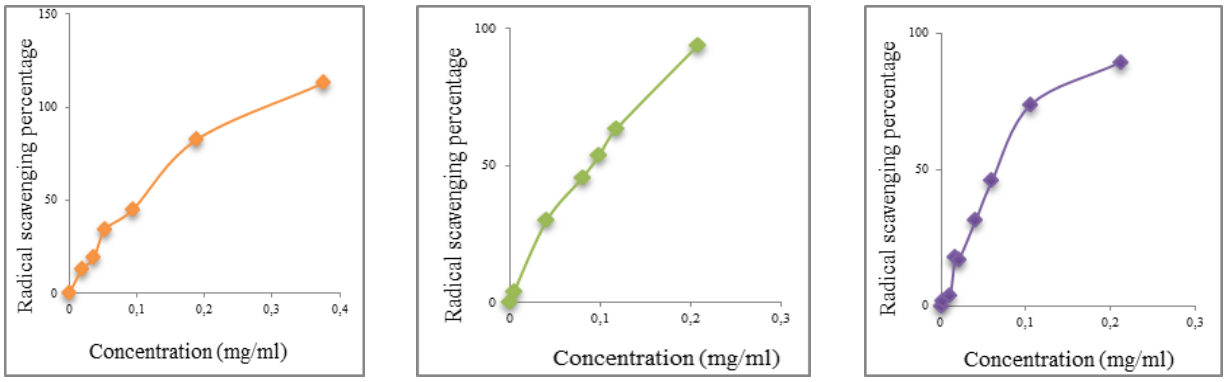



***Figure 3*. Content of condensed tannins of ethanolic, hydroethanolic and aqueous extracts of the trunk bark of *X. americana*.**




Ethanolic extract Hydroethanolic extract Aqueous extract

**Figure 4. Antiradical activity of trunk bark extracts of *X. americana***




Ethanolic extract Hydroethanolic extract Aqueous extract

**Figure 4. Antiradical activity of trunk bark extracts of *C. arborea***

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