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# Phytoconstituents, antimicrobial and antioxidant properties of the leaves of *Persea americana* Mill cultivated in Ghana

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**The leaves of *Persea americana* are widely used for ethno-medicinal purposes worldwide. This study assessed the antimicrobial and antioxidant activities of the methanol, ethyl acetate, chloroform and petroleum ether leaves extracts of *P. americana*. Extracts displayed variable antimicrobial activities that were microorganism-specific. The methanolic extract displayed the most potent antimicrobial activities with the largest zones of inhibition (0-1.8 mm) in the agar diffusion assay and with the lowest minimum inhibitory concentration (MIC) in the broth dilution assay against a panel of microorganisms that included *Bacillus subtilis, Escherichia coli, Salmonella typhi, Streptococcus aureus, Pseudomonas aeruginosa and Candida albicans.* The ethyl acetate extract exhibited the most potent antioxidant potential with the lowest $EC_{50}$ of $4.15x10^{-30}$ g/ml for the peroxide radical scavenging activities. The data supports the ethnomedicinal use of the leaves of *P. americana* for the management of infections and for other symptoms whose etiology may be linked to oxidative stress.**

**Key words:** *Persea americana*, antioxidant, antimicrobial, phytochemical screening, minimum inhibitory concentration.

## INTRODUCTION

There is emerging general and scientific interest these days for discovering phytochemicals as distinct options for the synthetic substances that are normally used in food, pharmaceutical and cosmetic industries. This interest for natural products as alternatives to synthetic products has been used by medical doctors, scientific researchers, as well as by general public (Borchers et al., 2000; Truiti et al., 2003). This thought is bolstered by the buyer's worry about the safety of items containing synthetic chemicals in light of the fact that such molecules are suspected to bring about or advance negative health effects (Rodríguez-Carpena et al., 2011).

Therapeutic plants have kept on drawing in consideration worldwide in the quest for powerful antimicrobial drugs that can battle resistant pathogens that have rendered numerous conventional medications out of date in the treatment of diseases (Cox, 1990). Numerous medications utilized in medicine are acquired

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from plants (Idris et al., 2009). The most active of these bioactive constituents of plants are alkaloids, tannins, steroids, terpenoids and phenolics.

The important function of antioxidants is suppressing the oxidation of different molecules by restraining the initiation or propagation of oxidizing chain reactions by free radicals and, accordingly, diminishing oxidative harm (Frankel and Meyer, 2000). Antioxidants act in different ways, which incorporate complexation of redox-catalytic metal particles, scavenging of free radicals, and decomposition of peroxides. Using different methods for the investigation of antioxidant action of food related extracts permit a complete screening of their plausible antioxidant activities (Frankel and Meyer, 2000). Unrefined extracts of herbs, natural products, flavors, and other plant materials rich in phenolics are of expanding enthusiasm for the food industry on the grounds that they hinder the oxidative degradation of lipids, and thus enhance the quality and nutritional estimation of foods (Bastida et al., 2009; Ganhão et al., 2010).

Phenolic compounds in plants function as antioxidants due to their redox properties. They are therefore useful as reducing agents, hydrogen donors, free radical quenchers, and metal chelators. A lot of antioxidants have already been extracted and isolated from different parts of plants and plant materials, such as oil seeds, cereal crops, vegetables, fruits, leaves, roots, spices and herbs (Alothman et al., 2009; Badu et al., 2012; Garcia-Alonso, 2004; Kähkönen et al., 1999; Velioglu et al., 1998).

*Persea americana* originated from southern Mexico, however, they are now grown worldwide. Countries where *P. Americana* is cultivated include Australia, South Africa and Spain (Rodríguez-Carpena et al., 2011). Because of the high demand for *P. Americana* fruit worldwide owing to its high nutritional value and reported health benefits, including anticancer activity, the food industry has grown tremendous interest in processing this crop and enhancing its value (Lu et al., 2005). *P. americana* leaves' extracts have been used as analgesic, anti-inflamatory, hypoglycaemic, anticonvulsant, antidiabetic and vasorelaxant among other therapeutic uses (Adeyemi et al., 2002; Antia et al., 2005; Gondwe et al., 2007; Ojewole and Amabeoku, 2006; Owolabi et al., 2005).

A few studies have focused on the phytochemical composition of *P. Americana* (Torres et al., 1987). *P. americana* leaves have been reported to possess anti-inflammatory (Adeyemi et al., 2002), antifungal (Prusky et al., 1991) and antibacterial activities (Gomez-Flores, 2008). There is scarce information available in the literature about the total phenolic content and antioxidant capacity of the leaves (Owolabi et al., 2010; Yasir et al., 2010), pulp (Alothman et al., 2009) and residues from *P. Americana* fruit (Wang et al., 2010). Exploiting the phytochemical content of *P. Americana* waste materials such as leaves, peel and seed may lead to new food products of enhanced quality, and that would have a significant impact on both the *P. americana*and the processed-food industries. Although, the antimicrobial and antioxidant properties of *P. Americana* have been widely reported, there has been no report on the species grown in Ghana.

The aim of the present study was to determine the phytoconstituents of the leaves, the antimicrobial and antioxidant potential of different leaves' extracts (ethyl acetate, chloroform, petroleum ether and methanol) of *P. americana* cultivated in Ghana.

## MATERIALS AND METHODS

### Chemicals

All chemicals and reagents used for the present work were purchased from Sigma Chemicals (Huge Ltd, Accra, Ghana). All the solvents used for extraction were suitable for industrial food use and were used as received without any further purification or treatment.

### Sample collection and preparation

Fresh leaves of *P. americana* Mill were collected from the Kumasi metropolis in The Ashanti region of Ghana. The leaves were plucked from different *P. Americana* trees which weighed a total of 1.5 kg. The leaves were washed with distilled water to remove dirt settled on it and air-dried for seven days. The dried leaves were pulverized into powder using a mill. The powder was stored in a transparent air-tight container, labeled with a permanent marker and stored at room temperature.

### Phytochemical Screening

Phytochemical screening was carried out on the powdered sample to determine the presence of eleven pharmacologically active phytochemicals in the leaves using standard methods described by Trease and Evans (1989). The phytochemicals determined were tannins, saponins, general glycosides, terpenoids and steroids, carotenoids, coumarins, alkaloids, anthraquinones, cyanogenic glycosides, flavonoids and anthraquinone glycosides.

### Extraction of phytochemicals from *Perseaamericana*leaves

A soxhlet extraction method was used for the extraction of phytochemicals from the *P. Americana* leaves. 400 ml each of methanol, chloroform, ethyl acetate and petroleum ether was used to extract from 50 g each of the powdered plant sample for 10 h. The solvent was then removed with a rotary evaporator after which the extracts were dried by slow evaporation at room temperature.

### Antimicrobial analysis

The methanol, chloroform, ethyl acetate and petroleum ether leaves extracts were tested against *Escherichia coli, Salmonella typhi, Streptococcus aureus, Candida albicans, Pseudomonas aeruginosa and Bacillus Subtili*s to ascertain their zone of inhibition and minimum inhibitory concentrations. These organisms were chosen because they are the common gram positive, gram negative

**Table 1.** Phytoconstituents of *P. americana* leaves.

| Test | Result |
|---|---|
| Glycosides | Present |
| Alkaloids | Present |
| Tannins | Present |
| Saponins | Present |
| Flavonoids | Present |
| Terpenoids and steroids | Present |
| Cyanogenic glycosides | Absent |
| Coumarins | Absent |
| Anthraquinones | Absent |
| Anthraquinone glycosides | Absent |
| Carotenoids | Absent |

and fungus.

#### Zone of inhibition

The zone of inhibition was determined using the nutrient agar method. Twenty-four (24) petri-dishes with each petri-dish corresponding to one test organism for each extract and labeled in allthe four different concentrations of the plant extracts were used. A 20 ml of nutrient agar in the petri-dish labeled for the organism. The nutrient agar was allowed to solidify and wells created in them using the cork borer (6 mm). Each well was filled with its respective concentration of the plant extract and left for about one hour for complete diffusion of the extract within the nutrient agar. The petri-dishes containing the nutrient agar were then incubated between 37°C and 42°C for a period of 18 h after which the zone of inhibition was determined.

#### Antimicrobial activity index

Antimicrobial index (AI) for methanol, ethyl acetate, chloroform, and petroleum ether extracts of *P. Americana* leaves were calculated as the mean value of the antimicrobial activity obtained against the sum of all individual microorganisms. Weight age was assigned to activity of extracts against each microbe. For zone of inhibition up to 10 mm, a weightage of 1 was given and that ranging from 11 to 20 mm, weightage of 2 was assigned. For zone of inhibition greater than 20 mm, weightage of 3 was assigned and for no antimicrobial activity, weightage of zero was assigned. The sum total of weightages obtained by each extract divided by the total number of pathogens tested gave the AI of the extract.

#### Antioxidant assay by hydrogen peroxide decomposition

The antioxidant activity of *P. Americana* leaves was determined using the hydrogen peroxide decomposition method by iodometric titration. A 2 ml, 4 ml and 8 ml aliquots of the methanol extract was prepared by dissolving 1 g of extract in 100 ml distilled water, added to 8 ml of 17 mM $H_2O_2$ solution in three different conical flasks. The mixture in each conical flask was mixed by gentle swirling motion. A 25 ml of distilled water was added to 1 ml of the mixture from each conical flask to slow the consumption of $H_2O_2$ by the extract at 60 s interval for a period of 4 min. A 2.2 g of KI and 10 ml of 2M $H_2SO_4$ were added to each of the mixtures in the conical flasks and the liberated iodine gas ($I_2$) titrated against 0.0519M standard sodium thiosulphate solution using starch as indicator. A blank was also titrated against standard sodium thiosulphate and the titre subtracted from that of the extract before its concentration was determined. The decomposition of $H_2O_2$ for three different concentrations of the extract was plotted against time of decomposition to ascertain the antioxidant activities of the extracts.

#### Statistical analysis

A one way analysis of variance (ANOVA) was done using Microsoft excel software to establish the presence or absence of variability between the antimicrobial activities of various extracts and same extracts at different concentrations. Student's t-test analysis was carried out for analyzing the results. P values at $<0.05$ were considered for describing the significant levels.

## RESULTS AND DISCUSSION

### Phytoconstituents of *P. americana* leaves

Phytochemical screening of the leaves of *P. Americana* showed the presence of tannins, saponins, terpenoids and steroids, alkaloids, flavonoids and glycosides as presented in Table 1. The presence of these phytochemicals has pharmacological and medicinal importance to humans (Yasir et al., 2010). For example, alkaloids can act as antimalarial, anticancer, antiasthma and antibacterial pharmacological constituents in humans. Tannins on the other hand have been used to combat diarrhea (Idris et al., 2009). The presence of tannins also enhances the antioxidant properties of the *P. americana* (Alothman al., 2009). Saponins have gained grounds as a dietary supplement and nutraceutical (Akinpelu et al., 2014). Saponins have also been used to lower blood cholesterol level and also as an anticancer agent. Furthermore, its amphipathic properties promote the penetration of proteins through the cell membranes. Glycosides are known for their antibiotic properties. The therapeutic properties of *P. Americana* based on the phytoconstituents cannot be overlooked (Cox, 1990). The phytoconstituents obtained in this study can be compares well with other literature reports (Owolabi et al., 2010; Yasir et al., 2010).

### Antimicrobial activity of the leaves' extract of *Perseaamericana*

The antimicrobial activity of the leaves extract of *P. americana* was tested against five bacterial strains and one fungal strain. The microorganisms were *B. Subtilis, C. albicans*, *P. aeruginosa*, *S. aureus*, *S. typhi* and *E. coli*. Antimicrobial activities were assessed by determining the zone of inhibition (measured in mm) of the various extracts against the test microbes (Figure 1). The size of this zone depends on the effectiveness of the extract against the growth of microbes. Usually, at higher

**Table 2.** Antimicrobial activity index of extracts of *Perseaamericana*leaves at different concentrations.

| Concentration (%) | Methanol extract | ethyl acetate extract | Chloroform extract | Petroleum ether extract |
|---|---|---|---|---|
| 1.25 | 0.50 | 0.17 | 0.33 | 0.00 |
| 2.5 | 0.83 | 0.67 | 0.50 | 0.00 |
| 5 | 1.00 | 1.00 | 0.67 | 0.50 |
| 10 | 1.00 | 1.00 | 0.67 | 0.83 |

**Table 3.** Minimum inhibitory concentration.

| Microbe | Minimum inhibitory concentration (%) | | | |
|---|---|---|---|---|
| | Methanol extract | Ethyl acetate extract | Chloroform extract | Petroleum ether extract |
| *B. Subtilis* | $1.56 \times 10^{-6}$ | 0.93 | 0.62 | 1.98 |
| *C. albicans* | 0.036 | 1.48 | 1.05 | 6.29 |
| *P. aeruginosa* | 0.942 | 0.95 | No inhibition | 0.196 |
| *S. aureus* | 15.96 | 0.92 | No inhibition | 1.34 |
| *S. typhi* | $3.16 \times 10^{-5}$ | 0.093 | 0.077 | 1.34 |
| *E. coli* | 1.702 | 1.48 | 1.67 | No inhibition |

concentrations of the antibiotics, larger zones are created. The methanol extract was active against *S. typhi, C. albicans, and B. Subtilis* at low concentrations (1.25%). The extract showed no antimicrobial activity against *E. coli* at concentration ≤ 2.5%.

The methanol extract was very potent against *B. Subtilis* whereas very low potency was recorded for *E. coli*. Although, these two microbes are rod-like, the outer membrane of the *E. coli* which is a gram negative bacterium is able to inhibit the antimicrobial activity of the extract. The ethyl acetate extract was only potent against *S. typhi* at higher concentrations. Increasing the concentrations from 1 to 10 % increased the antimicrobial activity against all the microbes. The petroleum ether and chloroform extracts showed no antibacterial activity against *E. coli.* Generally, the polarity index of the solvent had a great impact on the antibacterial activity of the leaves extracts as it was observed that all the extracts showed decreasing antimicrobial activity in order of decreasing polarity index of the extracting solvent.

The methanol, ethyl acetate, chloroform and petroleum ether extracts of the leaves exhibited antimicrobial activity on all the test organisms producing zone of inhibition ranging from 0 to 1.8, 0 to 0.9, 0 to 0.7 and 0 to 0.4 mm respectively. These results are in good agreement with the antimicrobial activity of seed extracts of *P. Americana* on similar test organisms (Idris et al., 2009). The methanol extract inhibited the growth of *C. albicans* at all concentrations whereas for ethyl acetate, chloroform and petroleum ether extracts, the extent of inhibition was concentration dependent. That is, at lower concentrations no inhibitions were observed.

The antimicrobial activity index of extracts of *P. Americana* leaves at different concentrations was also investigated and is detailed in Table 2. Methanol extract of the leaves recorded the highest antimicrobial activity and achieved the highest activity index among all the extracts. The difference in the activity indices may be due to different phytoconstituents present in the individual extracts. This is because different solvents have different degrees of solubility for different phytoconstituents (Gopalakrishnan et al., 2012). With the exception of the petroleum ether extract, increasing concentration of the extract from 5 to 10% had no effect on the activity index.

The minimum inhibitory concentration (MIC) of the methanol, ethyl acetate, chloroform and petroleum ether extract of the leaves of *P. americana*was was tested against all six organisms. The solvents were chosen based on their polarity. Methanol has a polarity index of 5.1, ethyl acetate, 4.4; chloroform, 4.1 and petroleum ether, polarity index of 0.1. From Table 3, it was observed that the extracting solvent had a significant impact on the MIC of the microbes.

The low MIC for the extracts demonstrated the therapeutic potential of the phytoconstituents (Idris et al., 2009). The petroleum ether extract showed a higher MIC against *B. subtilis*, *C. albicans* and *S. typhi* than the other solvent used. It however, showed no inhibition against *E. coli.* This is in agreement with the zone of inhibition for the petroleum ether extract at various concentrations as high MIC indicates low antimicrobial activity.

## Antioxidant properties of *P. americana* leaves

In humans, oxidative stress results from a decrease in antioxidant potential or an increase in the production of oxygen radical. These are capable of altering the structure

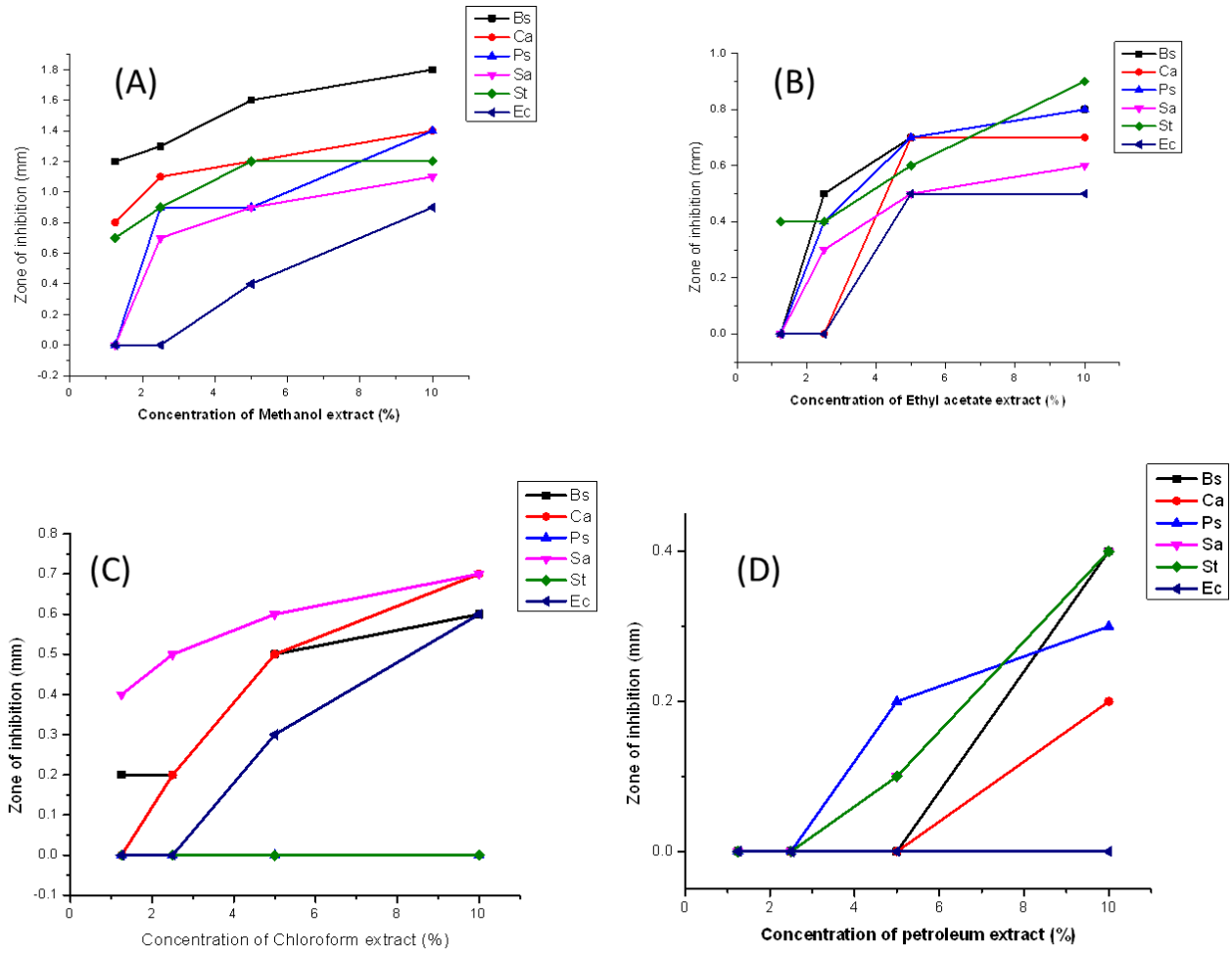


**Figure 1.** Zone of inhibition of micro-organisms against (A) methanol, (B) ethyl acetate, (C) chloroform and (D) petroleum ether extracts of *P. Americana* leaves. Bs, Ca, Ps, Sa, St and Ec represent *B. subtilis, C. albicans, P. aeruginosa, S. typhi, S. aureus and E.coli* respectively.

structure and functions of many biomolecules. *P. Americana* leaves' extracts have shown strong antioxidant activities (Figure 2). Generally, for all the extracts, increasing concentration corresponded to an increase in their antioxidant properties. There were however, no significant differences between % $H_2O_2$ decomposition at different concentrations of a particular extract $P>0.05$ and different extracts of similar concentration. The petroleum ether extract of the leaves showed no % $H_2O_2$ decomposition at all the concentrations used. This indicates no antioxidant activity of the extract.

The methanol extract showed an increase in the % $H_2O_2$ decomposition with respect to increasing concentration of the extract at constant time. However, at lower concentrations of the extract (0.02 g/ml), increasing time from 60 to 120 seconds resulted in a constant antioxidant activity. Increasing concentration of the extract from 0.02 to 0.08 increased the % $H_2O_2$ decomposition at constant time. Also, for all the three different concentrations used, increasing time corresponded at an increase in the % $H_2O_2$ decomposition. At low concentration of the extract (0.02 g/ml), a constant % $H_2O_2$ decomposition was recorded when the time was increased from 60 to 120 s.

At any particular concentration of the ethyl acetate extract, increasing time resulted in an increase in the % $H_2O_2$ decomposition. At a concentration of 0.08 g/ml, increasing time from 60 to 240 s resulted in a proportional increase in the % $H_2O_2$ decomposition. Comparing 0.02 and 0.04 g/ml concentrations of the ethyl acetate extract, the 0.04 g/ml concentration showed increase in % $H_2O_2$ decomposition when the time was increased from 60 to 120 s.

However, increasing the time further from 180 to 240 s resulted in a decrease in the% $H_2O_2$ decomposition as compared to the 0.02 extract concentration. Of all the extracts, ethyl acetate extracts showed the highest

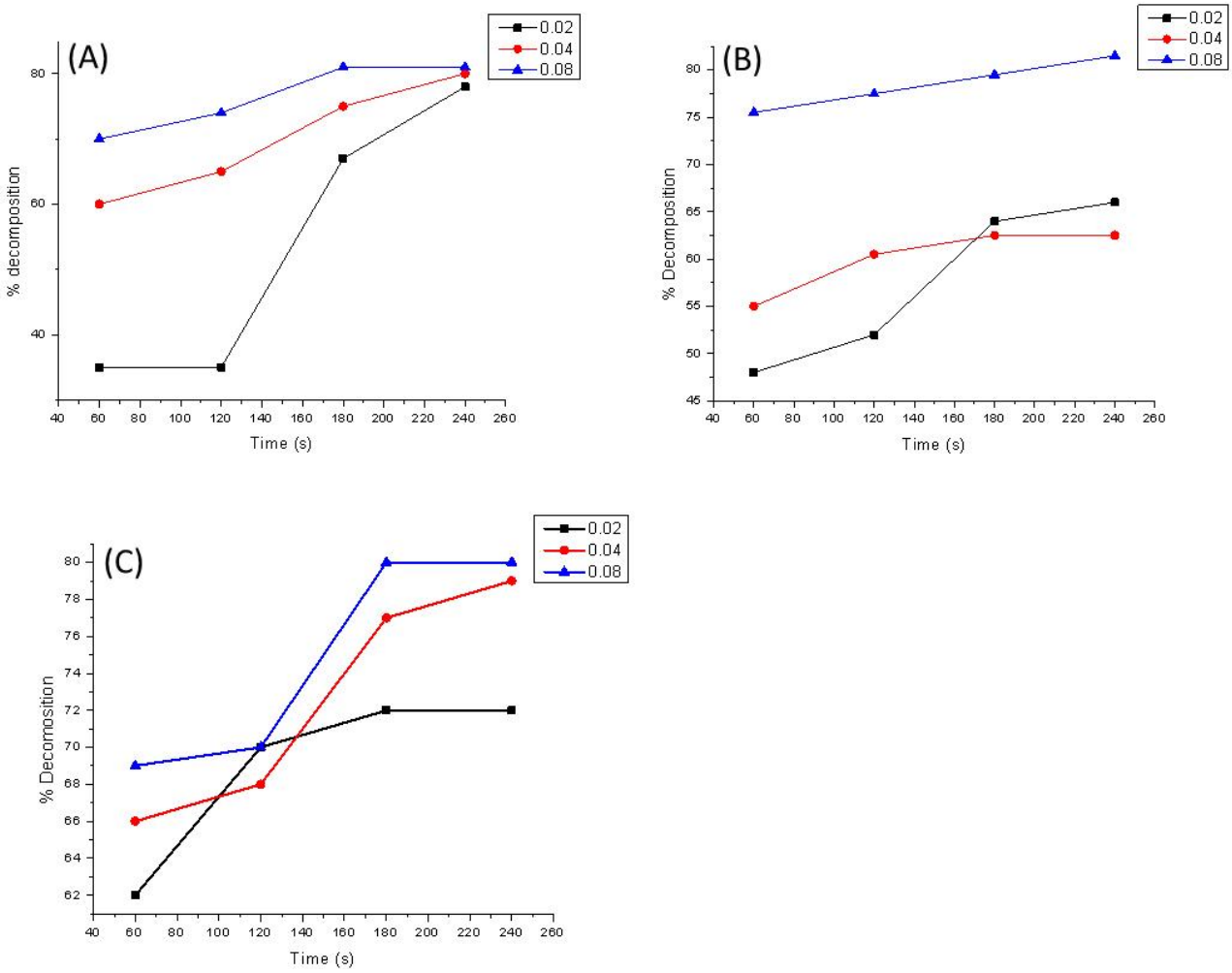


**Figure 2.** % $H_2O_2$ decomposition for (A) methanol, (B) ethyl acetate and (C) chloroform extracts.

antioxidant activity with $EC_{50}$ of $4.15 \times 10^{-30}$ g/ml. Methanol extract followed with $EC_{50}$ of $5.52 \times 10^{-10}$ g/ml and chloroform extract with $EC_{50}$ of $6.57 \times 10^{-5}$ g/ml. The antioxidant property of the leaves extract is in good agreement with earlier report by Owolabi et al. (2010).

This study used the % $H_2O_2$ decomposition method to determine the antioxidant of the leaves of *P. americana* and concluded that the leaves of *P. americana* contain antioxidant which can help prevent stress related diseases. Also, Ikpeme et al. (2014) evaluated the antioxidant efficacy of fresh and dried fruits of *P. Americana* and reported that the fresh fruit is more efficient that the dry fruit.

## Conclusion

Phytochemical screening of the leaves of *P. americana* showed the presence of tannins, flavonoids, saponins, terpenoids, steroids, alkaloids and glycosides.

The antimicrobial activities of methanol, ethyl acetate, chloroform and petroleum ether extracts of *P. Americana* leaves have been investigated. The extracts were tested against *B. subtilis, E. coli, S. typhi, S. aureus, P. aeruginosa and C. albicans.* All the extracts were potent against the test organisms with the methanol extract exhibiting the highest zone of inhibition.

Also, the antioxidant properties of the extracts have been investigated. All the extracts except petroleum ether showed antioxidant activities. Of all the extracts, methanol extracts showed the highest antioxidant activity. This research has confirmed the antimicrobial and antioxidant properties of the leaves' extracts of *P. americana*.

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## Conflicts of interest

The authors have none to declare.

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