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# Variation of antioxidant and antibacterial activities of ethanolic extracts of propolis in three bee-keeping agro-ecological zones of Uganda

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**Propolis is a resinous plant material collected by bees to defend their colony. This study evaluated the antibacterial and antioxidant activities of ethanolic extracts of Ugandan propolis in three bee-keeping agro-ecological zones. Antibacterial assays were performed on two Gram-positive (*S. aureus* and *S. pneumoniae*) and two Gram-negative (*E. coli* and *P. aeruginosa*) bacteria within a concentration range of ~1.6 to 100 mg/ml. Antioxidant assays were conducted spectrophotometrically on the basis of DCPIP reduction and the attendant decrease in absorbance at 605 nm wavelength. All extracts showed antibacterial activity against *S. aureus* with MICs ranging from 2.8 to 200 mg/ml, but *P. aeruginosa* displayed susceptibility only for samples from Western Highlands (MIC = 9.5 mg/ml). Of the Gram-negatives, *E. coli* was the more susceptible organism (MICs 5.7-31.5 mg/ml), but *S. pneumoniae* was susceptible only to samples from mid northern and Lake Victoria Crescent (MIC 34.6 mg/ml). Samples from Mid Northern region exhibited the highest antioxidant activity (mean ± SD activity equivalent to 20.4±4.3 µg of ascorbic acid per mg of extract), while those from Western Highlands exhibited the lowest (mean ± SD activity equivalent to 8.9 ± 2.5 µg of ascorbic acid per mg of extract). The antibacterial and antioxidant activities of propolis varied within and, more significantly, between the agro-ecological zones. Taken together, these results highlight the potential of Ugandan propolis as an antioxidant and antibacterial agent. Strategic selection of hive localities in zones that offer the best output in propolis should be a priority for bee-farmers.**

**Key words:** Propolis, agro-ecological zones, 2,6-dichlorophenolindophenol (DCPIP), antibacterial, antioxidant, ascorbic acid, *Apis mellifera*.

## INTRODUCTION

Propolis is one of the main products of honeybees (*Apis mellifera*) to which their evolutionary success has been widely attributed, giving them the ability to exploit virtually any habitat on earth. The bees obtain propolis from resinous exudates of tree buds and craft it into a finished product which invariably takes a wax-like appearance. It is used both as a building material and a defense tool for the hive, owing to its mechanical and biological properties (Bankova et al., 2014). As a building material, propolis is used by bees to seal gaps and small holes in the hive,

giving the latter a smooth but sticky finish. Defensively, it is used to mummify or embalm intruders that have just met their death in the hive upon being stung (Siheri et al., 2017). Thus, using propolis, the bees are able to secure their hive as a safe shelter free of both macro- and micro-organisms. This helps to maintain the hive's sanitary hygiene thereby safeguarding the health and integrity of the colony. Having propolis as a first line of defense also minimizes the likelihood of bees having to heroically sacrifice themselves through the act of stinging in defense of the colony.

Since the 1960's, there has been increased focus on propolis-related research, inspired by a broader interest in natural products as a whole (Kuropatnicki *et al*., 2013). Several of the studies conducted on propolis so far have revealed that it has antioxidant (Vargas-Sanchez *et al*., 2014, Zhang *et al*., 2013), antiviral (Yildirim *et al*., 2016), antibacterial (Auamcharoen and Phankaew, 2016, Nina *et al*., 2016), anti-parasitic (Alday-Provencio *et al*., 2015), and cardio-protective (Daleprane and Abdalla, 2013) activities. The use of bee products such as honey, propolis, royal jelly and beeswax in pharmaceutical and cosmetic formulations is well established (UEPB, 2005). Research shows that, of all the bee products, propolis has the strongest antioxidant effect and may be of paramount importance in managing some oxidative-stress-related disease states (Nakajima et al., 2009). Most of these past studies on propolis have been conducted *in vitro* on temperate and Brazilian samples, the latter arguably being the most documented thus far. Tropical African propolis, particularly from Eastern Africa, remains largely uncharacterized.

There is a general consensus on the chemical complexity of propolis and how this complex chemical profile correlates with diverse spatiotemporal aspects of sample provenance, such as climate, geographic factors, types of vegetation foraged by the bees, bee species, time of collection, and season of the year (Seidel et al., 2008; Nina et al., 2015; Massaro et al., 2013; Muli and Maingi, 2007). These factors, by influencing the diversity of compounds, and their proportions, in propolis, subsequently lead to its varying biological effects (Siheri et al., 2016). The main constituent bioactive compounds belong to such diverse chemical classes as flavonoids, phenylpropanoids, terpenes, stilbenes, lignans, coumarins, and their prenylated derivatives (Huang et al., 2014).

Generally, Uganda boasts a warm tropical climate with an average temperature range of 25 to 29°C (77-84°F) and annual rainfall of 1,000 to 1,500mm. In the south, the year is split into four seasons, where dry months (January to February and June to August) alternate with rainy ones (March to May and September to December, respectively). In northern Uganda, the seasons are more pronounced, and there is only one wet season (April to October) and one dry season (November to March), and there the vegetation is sparse and consists of acacia trees, cacti, and shrubs (US Marine Corps Uganda, 2014).

The country is divided into 10 different agro-ecological zones classified on the basis of distinct vegetation type, elevation and climatic conditions. A vibrant apiary business obtains throughout Uganda but more so in the Western Highlands, Lake Victoria Crescent, Mid-Northern region and West Nile (Kajobe et al., 2009), and in these zones beekeepers mostly farm the native East African lowland honeybee (*A. mellifera scutellata)*. According to the Uganda National Apiculture Development Organisation's (TUNADO) 2015 report, propolis was the third most traded bee product in Uganda and is employed for medicinal use as an antioxidant or antimicrobial (Runyoro et al., 2017). However, there is no sufficient locally derived scientific data either to confirm these properties or to justify the therapeutic claims. In addition, such data would need to reveal how the biological properties vary in propolis samples from the various beekeeping regions of the country in order to facilitate optimization of apiary localization and improve business productivity.

In studies to elucidate the chemical structures of active compounds present in propolis, several methods have been used for their extraction, isolation and purification. The commonly used extraction methods include maceration with or without sonication, soxhlet extraction, and microwave-assisted extraction (Khacha-Ananda et al., 2013). On the other hand, contemporary methods for isolation and purification rely on chromatographic techniques, such as column chromatography, medium pressure liquid chromatography, and preparative high performance liquid chromatography (HPLC) (Zhang et al., 2014; Siheri et al., 2014). The extraction method used has a significant effect on the chemical composition, and thus biological activity, of propolis extracts. Given its moderate polar properties, ethanol offers a convenient option when preparing propolis extracts since it enables higher yields of low-wax extracts rich in biologically active compounds (Taddeo et al., 2016).

This study employed *in vitro* antibacterial assays and spectrophotometric analysis to assess the antibacterial and antioxidant properties, respectively, of propolis sourced from three of the four main beekeeping agro-ecological zones of Uganda, namely, mid-Northern, Lake Victoria crescent, and Western highlands. The mid-Northern agro-ecological zone straddles the districts of

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Lira, Apac, Kitgum, Gulu, and Pader; Lake Victoria crescent covers the districts of Masaka, Mpigi, Luweero, Kampala, Mukono, Kayunga, Wakiso, Kiboga, Nakasongola, Kalangala, and Mubende. On the other hand, Western highlands is composed of the districts of Bushenyi, Kasese, Bundibugyo, Kamwenge, Kyenjojo, and Kabarole. Identification of agro-ecological provenances associated with highly active propolis could be an incentive for commercial apiculture as a means of poverty alleviation and socioeconomic development.

## MATERIALS AND METHODS

### Study design

In this study, ethanolic extracts of bee propolis from the three selected agro-ecological zones of Uganda were analyzed for their antioxidant and antimicrobial activities using spectrophotometric analysis and *in vitro* antibacterial assays, respectively.

### Study samples

The propolis samples were collected from two apicultural farms from each of the three selected agro-ecological zones of Uganda, namely, mid-Northern (Gulu, Northern Uganda; $N_1$, $N_2$), Lake Victoria crescent (Masaka, Central Uganda; $C_1$, $C_2$) and Western highlands (Kabarole, Western Uganda; $W_1$, $W_2$). The collected samples were securely packaged in stoppered plastic containers and transported to the Pharmaceutical Chemistry/Analysis laboratory at Mbarara University of Science and Technology (MUST) for extraction and analysis. Extracted samples were stored at -20°C until required for analysis.

### Extraction of propolis

Approximately 30 g of each of the previously chopped samples were separately mixed with 70% (v/v) ethanol in water in a 100 mL beaker and agitated continuously at 120 cycles/min at room temperature using a reciprocating shaker (IKA HS 260 Basic, Germany) in a dark room for 72 h. Thereafter, the samples were filtered and the filtrate evaporated to dryness at room temperature under a slow air current generated from an electric fan.

### Sample preparation

Approximately 200 mg/mL stock solutions of each of the propolis samples were separately prepared in distilled water and vortex-mixed. These solutions were then subjected to two-fold serial dilutions with brain heart broth to attain subsequent concentrations of 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 mg/mL, respectively. All solutions were mixed thoroughly at each stage before the next dilution to ensure homogeneity.

### Determination of minimum inhibitory concentrations (MICs) of extracts

Pure standard samples of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Streptococcus pneumoniae* ATCC 49619 were obtained from the microbiology laboratory of Mbarara Regional Referral Hospital (MRRH) where antibacterial assays were all conducted. The 0.5 McFarland standards of bacterial suspensions of each of the study microorganisms were vortex-mixed (Benchmark, UK) and later measured using the Densimat (Biomerieux, Italy). Exactly 200 µL of each of the eight assay solutions (at concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 mg/mL) of propolis extracts in brain heart broth were transferred to previously bored wells (8 mm diameter; 5 wells per plate; 2 plates per microorganism) on Mueller Hinton Agar (MHA) plates inoculated with *S. aureus* and incubated at 35 to 37°C for 48 h. The same procedure was repeated for *E. coli* and *P. aeruginosa*. For *S. pneumoniae*, 200 µL of the assay solutions were transferred to wells on chocolate agar plates inoculated with the organism and incubated at 35 to 37°C in a candle jar (10% $CO_2$) for 48 h. Wells containing 200 µL of ciprofloxacin (2 mg/ml) and a two-fold dilution of brain heart broth in distilled water were used as positive and negative controls, respectively.

For the first 24 h, the plates were incubated in their normal upright position to enable diffusion of the extract into the agar and also prevent the extract from leaking. But for the next 24 h, the plates were flipped to prevent condensed water from sipping back into the agar. The diameters of the zones of inhibition (ZOI) were then measured after the incubation period and adjusted by subtracting the diameter of the wells (8 mm). The adjusted values were then used to determine MIC by plotting graphs of these adjusted values of ZOI against log concentration of the propolis sample extract as previously reported (Kronvall, 1982). The MIC was computed by taking the antilog of the x-intercept.

### Determination of minimum bactericidal concentrations (MBCs) of extracts

To determine MBC, 1 mL solutions of each of the seven serial dilutions of propolis in brain heart infusion broth were each inoculated with 200 µL of 0.5 McFarland standards of the bacterial suspensions. The tubes were then incubated at 35°C for 48 h. The tubes were sub-cultured onto sterile plates of MHA for *S. aureus*, *E. coli* and *P. aeruginosa*, and chocolate agar for *S. pneumoniae*. Thereafter, the plates were incubated at 35°C for 24 h. Following the incubation, the lowest serial dilution that did not reveal any bacterial growth was taken as the MBC.

### Spectrophotometric determination of antioxidant activity based on DCPIP reduction

A 20 mM stock solution of 2,6-dichlorophenolindophenol (DCPIP) was prepared by dissolving an accurately weighed sample of approximately 290.08 mg in 50 ml of distilled water. A series of 8 calibration solutions of DCPIP in the range of 1 to 8 mM were then prepared from the stock solution at 1-point intervals by dilution with distilled water. Based on a previously reported wavelength maximum ($\lambda_{max}$) of 600 nm by Brugger et al. (2014), a wave length scan was performed on the 5 mM calibration solution of DCPIP in the range of 500 to 700 nm to reveal an experimental $\lambda_{max}$ at 605 nm. The absorbances of the 8 standard calibration solutions were then measured with a UV-Visible spectrophotometer at the $\lambda_{max}$ of 605 nm. A standard curve of absorbance against concentration was plotted and the molar extinction coefficient of DCPIP was derived from the slope.

### Validation of the linearity of DCPIP absorbance reduction by standard ascorbic acid

A 1 mM solution of ascorbic acid was prepared by accurately weighing approximately 0.0176 g of ascorbic acid and dissolving it in 100 ml of distilled water. An aliquot of the 1 mM ascorbic acid

solution was used to zero the spectrophotometer and the initial absorbance of a 10 mM aqueous solution of DCPIP at $\lambda_{max}$ = 605 nm obtained. Then, 0.2 ml aliquots of the 1 mM ascorbic acid solution were successively added to 10 ml of the 10 mM DCPIP solution in a test tube, and each time taking the absorbance readings before returning the cuvette contents into the test tube. A linear regression analysis was then performed of the absorbance readings against volume of ascorbic acid added to prove linearity of response.

#### Evaluation of antioxidant activity of propolis extract

To test for antioxidant activity of the extracts, 30 mg of the propolis extracts were separately reconstituted in 15 ml of distilled water to obtain 2 mg/ml solutions. For each extract, four test tubes each containing 10 ml of 10 mM DCPIP standard solution were also separately prepared. Using the extracts as the blanks, the absorbance of the standard 10 mM DCPIP at $\lambda_{max}$ = 605 nm was initially recorded before adding 2 ml aliquots of the 2 mg/ml extract. The absorbances of the resultant solutions at $\lambda_{max}$ = 605 nm were then recorded. The resulting mean decreases in absorbance from the quadruplicate (n=4) measurements for each extract were taken to be a direct measure of the antioxidant capacity of the propolis samples and hence used for data analysis. The statistical differences between agro-ecological zones and between apicultural sites within the same zone were obtained through one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons (post-hoc) test and Student t-test, respectively, at the 0.05 alpha level using IBM® SPSS® version 20.

#### Ethical considerations

Ethical approval was obtained from the Department of Pharmacy and from the Faculty Research and Ethics Committee (FREC) of the Faculty of Medicine, Mbarara University of Science and Technology (MUST). Strict adherence to biosafety measures was observed in the use and disposal of culture plates to safeguard human life and the environment.

## RESULTS

### Antibacterial activity

The results revealed variability in antibacterial activities even among samples collected from the same agro-ecological zone. Generally, *E. coli* and *S. aureus* were the most susceptible organisms, while *P. aeruginosa* and *S. pneumoniae* were less susceptible to the propolis extracts. Regarding *E. coli*, sample $C_2$ produced the largest zone of inhibition (ZoI) (11 mm) and lowest MIC of 12.5 mg/ml where the ZoI was 2 mm (Table 1). Samples $W_1$ and $N_1$ did not show any inhibitory activity towards *E. coli* even at the highest concentration used in the assay (200 mg/ml). The largest zone of inhibition against *S. aureus* (7 mm) was produced by $N_1$ and $N_2$ at 200 mg/ml, while C1 showed the smallest zone of inhibition (3 mm) at the same concentration. The lowest MICs against *S. aureus* were produced by $N_1$ and $W_1$ samples, each at 12.5 mg/ml where the ZoI was 3 mm for either extract.

Of the three agro-ecological zones, only the samples from the Western highlands showed significant inhibitory activity against *P. aeruginosa*, with $W_2$ producing the largest zone of inhibition (13 mm) at 200 mg/ml and lowest MIC of 12.5 mg/ml (ZoI, 3 mm) against the target organism (Table 1).

The largest zone of inhibition against *S. pneumoniae* (15 mm) was given by $C_1$ at a concentration of 200 mg/ml, but the lowest MICs were produced by $N_1$ and $N_2$, each at 50 mg/ml. Samples $C_2$ and both samples from the western highlands ($W_1$ and $W_2$) did not show any observable inhibitory activity against *S. pneumoniae* in the concentration range used.

It is notable that whereas none of the samples produced observable inhibition at concentrations ≤6.25 mg/mL against any of the tested organisms, all samples except $N_1$ exhibited broad spectrum activities particularly at of 200 mg/ml. No sample showed activity against all of the four bacterial species in the concentration range employed for the assays. *S. aureus* was the most susceptible microorganism to the propolis extracts, while *P. aeruginosa* was the least susceptible.

### Variation of antibacterial activity within zones

In the Western highlands, both samples showed inhibitory activity against *S. aureus* and *P. aeruginosa* in the concentration range used, but $W_2$ showed a lower MIC than $W_1$ against both of these bacterial species. Unlike the $W_1$ extract, $W_2$ was additionally active against *E. coli*, although none of the extracts inhibited the growth of *S. pneumoniae*.

In the Lake Victoria crescent, both samples had inhibitory activity against *S. aureus* and *E. coli*; $C_2$ had a lower MIC than $C_1$ against both *S. aureus* and *E. coli*. In addition, $C_1$ was also active against *S. pneumoniae* unlike $C_2$, although none of the samples inhibited growth of *P. aeruginosa*.

In the mid northern agro-ecological zone, both samples ($N_1$ and $N_2$) had inhibitory activity against *S. aureus* and *S. pneumoniae*, respectively. However, $N_2$ had a lower MIC than $N_1$ against *S. pneumoniae*, while $N_1$ had a lower MIC than $N_2$ against *S. aureus*. $N_2$ was additionally active against *E. coli* although none of the samples showed activity against *P. aeruginosa* within the range of concentrations employed for the assay.

### Variation of antibacterial activity amongst zones

Propolis samples from all the agro-ecological zones had inhibitory activity against *S. aureus* with the mid northern sample having the greatest activity. At least one sample from each of the three agro-ecological zones had inhibitory activity against *E. coli* with Lake Victoria crescent showing the greatest activity.

The Western highlands zone had both of its samples showing activity against *P. aeruginosa* but neither the mid northern nor the Lake Victoria crescent zones had any

**Table 1.** Minimum inhibitory concentrations of ethanolic extracts of propolis from different agro-ecological zones of Uganda on four different microbial organisms, *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pneumoniae*.

| Activity | Agro-ecological zone | | | | | |
|---|---|---|---|---|---|---|
| | Mid Northern | | Lake Victoria crescent | | Western highlands | |
| Organism | $N_1$ | $N_2$ | $C_1$ | $C_2$ | $W_1$ | $W_2$ |
| *E. coli* | >200 | 31.5 | 6.12 | 5.66 | >200 | 5.79 |
| *P. aeruginosa* | >200 | >200 | >200 | >200 | 200 | 9.47 |
| *S. aureus* | 4.42 | 100 | 200 | 100 | 200 | 2.76 |
| *S. pneumoniae* | 36.18 | 32.99 | 200 | >200 | >200 | >200 |

**Table 2.** Minimum bactericidal concentrations of the ethanolic extracts of propolis from the three different agro-ecological zones of Uganda.

| Activity | Agro-ecological zones | | | | | |
|---|---|---|---|---|---|---|
| | Mid northern | | Lake Victoria crescent | | Western highlands | |
| Organism | $N_1$ | $N_2$ | $C_1$ | $C_2$ | $W_1$ | $W_2$ |
| *E. coli* | >200 | 100 | 100 | 50 | >200 | 100 |
| *P. aeruginosa* | >200 | >200 | >200 | >200 | >200 | >200 |
| *S. aureus* | >200 | 100 | >200 | >200 | >200 | >200 |
| *S. pneumoniae* | >200 | 100 | >200 | >200 | >200 | >200 |

samples with observable activity against this microorganism at ≤ 200 mg/ml concentrations of the extracts.

Both the mid northern and Lake Victoria crescent zones had at least one sample with activity against *S. pneumoniae*; mid northern had both of its samples showing greater activity than the sample from the Lake Victoria crescent. On the other hand, none of the samples from the Western highlands agro-ecological zone inhibited the growth of *S. pneumoniae.*

## Bactericidal activity of the propolis extracts

All propolis samples except $N_1$ and $W_1$ showed bactericidal activity against at least one of the three microorganisms, that is, *E. coli*, *S. aureus* and *S. pneumonia*, within the concentration range of the extracts used for the assays (Table 2). It was evident that *E.coli* was the most susceptible organism to the propolis extracts. However, no sample showed bactericidal activity against *P. aeruginosa* in the same concentration range making it the least susceptible organism. All samples that showed bactericidal activity had shown inhibitory activity towards the same organisms. For instance, all the four sample extracts that showed inhibitory activity against *E. coli* ($N_2$, $W_2$, $C_1$ and $C_2$) also demonstrated bactericidal activity, with $C_2$ having the lowest MBC (50 mg/ml). In addition, $N_2$ showed bactericidal activity against all the three microorganisms that had revealed susceptibility towards its inhibitory activity.

## Molar extinction coefficient of DCPIP and validation of Beer-Lambert's law in DCPIP reduction with ascorbic acid

The plot of absorbance of DCPIP versus concentration yielded a linear curve with $R^2$ = 0.994 (Figure 1). The molar extinction coefficient (ε) deduced from the slope of the graph was 174 litres per mole per centimetre. Addition of aliquots of 1 mM ascorbic acid into a fixed volume (10 ml) of the 10 mM DCPIP led to a gradual fall in absorbance values which when plotted against the cumulative volume of the standard ascorbic acid solution added yielded a highly linear relationship ($R^2$ = 0.9992; Figure 2). Thus, the extent of DCPIP reduction-induced discolouration was proportional to the volume of the standard antioxidant added to the solution.

## Antioxidant activity of propolis extracts

The means ± standard deviations of changes in absorbance of 10mM DCPIP after addition of each of the six propolis extracts from the three agro-ecological zones, measured in quadruplicate, are shown in Table 3. The change in absorbance values were converted into ascorbic equivalents (in micrograms) by initially dividing each value by the slope of the graph in Figure 2 to obtain the volume of 1 mM ascorbic acid that would produce

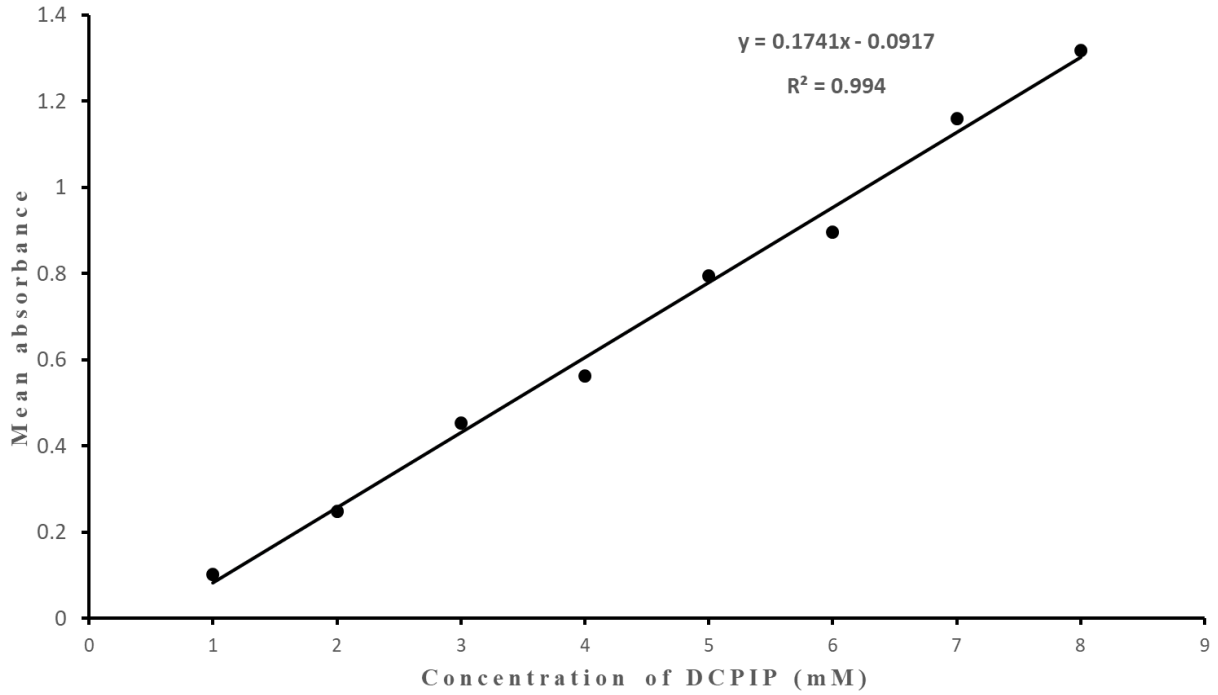


**Figure 1.** A plot of absorbance against concentration of DCPIP in mM. The eight standard DCPIP solutions were prepared in the range of 1 to 8 mM using distilled water. Absorbance measurements were made on a UV-Visible spectrophotometer at the $\lambda_{max}$ of 605 nm.

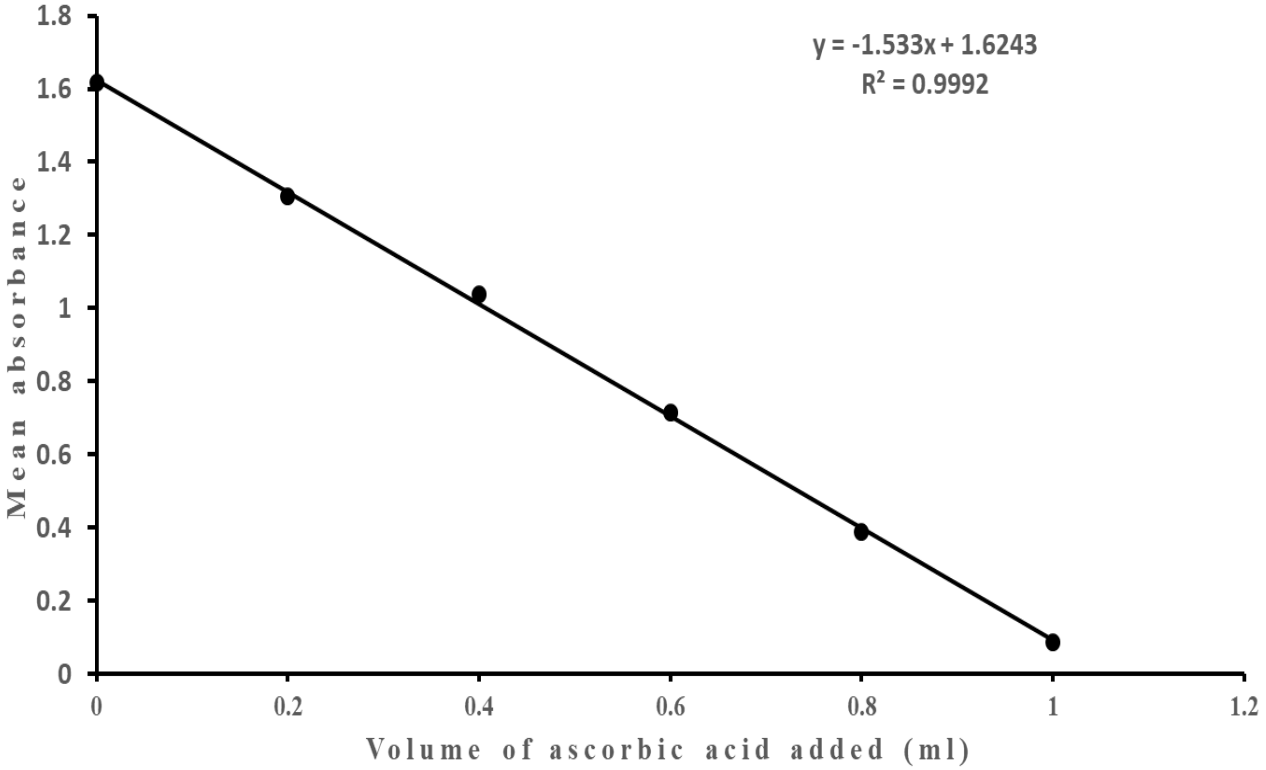


**Figure 2.** A plot of absorbance of 10mM DCPIP against volume of 1 mM standard ascorbic solution acid added. Aliquots of 0.2ml of a 1mM aqueous solution of ascorbic acid were successively added to 10ml of a 10 mM standard aqueous solution of DCPIP solution in a test tube, and each time taking the absorbance readings on a UV-Visible spectrophotometer at the $\lambda_{max}$ of 605 nm before returning the cuvette contents into the test tube.

**Table 3.** Comparison of the absorbance of 10 mM DCPIP after reduction by addition of each of the propolis samples from the three agro-ecological zones.

| Propolis sample | Change in absorbance (Mean ± Std. Dev.) | Ascorbic acid equivalent in µg per mg of extract | | Mean difference within zones (95% CI) | p-value |
|---|---|---|---|---|---|
| | | Within sites | Within zones | | |
| $N_1$ | 0.570 ± 0.0049 | 16.4±0.14 | 20.4±4.3 | -8.05 (-9.02, -7.08) | 0.000 |
| $N_2$ | 0.848 ± 0.0214 | 24.4±0.61 | | | |
| $C_1$ | 0.219 ± 0.0301 | 6.3±0.86 | 10.7±4.8 | -8.80 (-10.16, -7.43) | 0.000 |
| $C_2$ | 0.526 ± 0.0228 | 15.1±0.65 | | | |
| $W_1$ | 0.241 ± 0.0184 | 6.9±0.53 | 8.9±2.5 | -4.05 (-6.75, -1.35) | 0.016 |
| $W_2$ | 0.381 ± 0.0613 | 10.9±1.76 | | | |

The ascorbic equivalents were computed from the corresponding absorbance values at $\lambda_{max}$ = 605 nm of reduced 10 mM DCPIP solution based on the calibration curve in Figure 3. Each of the extracts was added as 2 ml aliquots of 2 mg/ml aqueous extracts to 10 ml solutions of 10 mM DCPIP. Antioxidant effect of the propolis sample extracts from the three agro-ecological zones based on reduction of 10mM DCPIP standard solution (n = 4); Values expressed as Means ± SDs.

such an absorbance change. The volumes of ascorbic acid were then converted into weight values based on the molecular weight of ascorbic acid (176.12 $gmol^{-1}$). Statistical differences between antioxidant activities between apicultural sites within the same agro-ecological zone, and those between agro-ecological zones were determined using the Student's t-test at an alpha level of 0.05. The changes in absorbance were used as a direct measure of the antioxidant capacity of the propolis samples.

The mid northern agro-ecological zone exhibited the highest mean antioxidant activity (equivalent to 20.4 µg of ascorbic acid per mg of extract) whereas the Western highlands exhibited the lowest mean antioxidant activity (equivalent to 8.9 µg per mg of extract). There was a significant difference ($p < 0.05$) in mean antioxidant activities between each of the apicultural sites within all of the agro-ecological zones (Table 3). Samples from Lake Victoria crescent ($C_1$ and $C_2$) showed the highest variation in antioxidant activity per mg of extract (equivalent to 8.8 µg of ascorbic acid), while samples from Western highlands ($W_1$ and $W_2$) showed the lowest variation (equivalent to 4.05 µg of ascorbic acid per mg of extract). From the multiple comparisons, there was a significant difference ($p < 0.05$) between mid-northern and the other two zones (that is, Western highlands and Lake Victoria crescent), but no significant difference ($p > 0.05$) was noticed between Lake Victoria crescent and Western highlands (Figure 3). The mid northern samples showed significantly higher antioxidant activity compared to samples from either Lake Victoria crescent or Western Highlands.

## DISCUSSION

### Antibacterial activity

Overall, all samples had the lowest MICs against *S. aureus*, making it the most susceptible microorganism. In a study of Brazilian propolis, Fernandes Jr et al. (1995) observed highest activity against Gram-positive bacteria particularly *S. aureus* compared to Gram-negative bacteria such as *E. coli* (Fernandes Jr et al., 1995). This higher susceptibility of *S. aureus* towards propolis was corroborated by studies from other countries (Kujumgiev et al., 1999; Sforcin et al., 2000; Marcucci et al., 2001; Gonsales et al., 2006).

*P. aeruginosa* was the least susceptible organism. This supports previous studies showing that propolis is less active towards Gram-negative compared to Gram-positive bacteria (Sforcin et al., 2000; Drago et al., 2000). It has been suggested that the resistance of Gram-negative bacteria could be due to the presence of efflux pumps preventing intracellular entry of propolis constituents (Garedew et al., 2004). The weak effect on Gram-negative bacteria may also be explained by the fact that propolis contains mainly plant-derived resin constituents and that resins are secreted by plants to protect mostly from Gram-positive pathogens (Garedew et al., 2004). It has also been reported that bees infected by Varroa mites (a common parasite which can destroy the hive) harbor predominantly Gram-positive bacteria (Bendel, 2002). Thus, in consistence with our findings, the antibacterial content of the propolis is probably more tailored towards Gram-positive bacteria.

Broad spectrum activity was defined by Ory and Yow (1963) as activity on the two major bacterial groups, Gram-positive and Gram-negative. In our study, all samples had broad spectrum activity except $N_1$ which only had activity against Gram-positive bacteria. Our propolis samples produced dissimilar results in their antibacterial activities; this dissimilarity in antibacterial activity of propolis from different regions has been documented before. For instance, Gonsales et al. (2006) reported that the ethanolic extract of Brazilian propolis was effective only against Gram-positive bacteria;

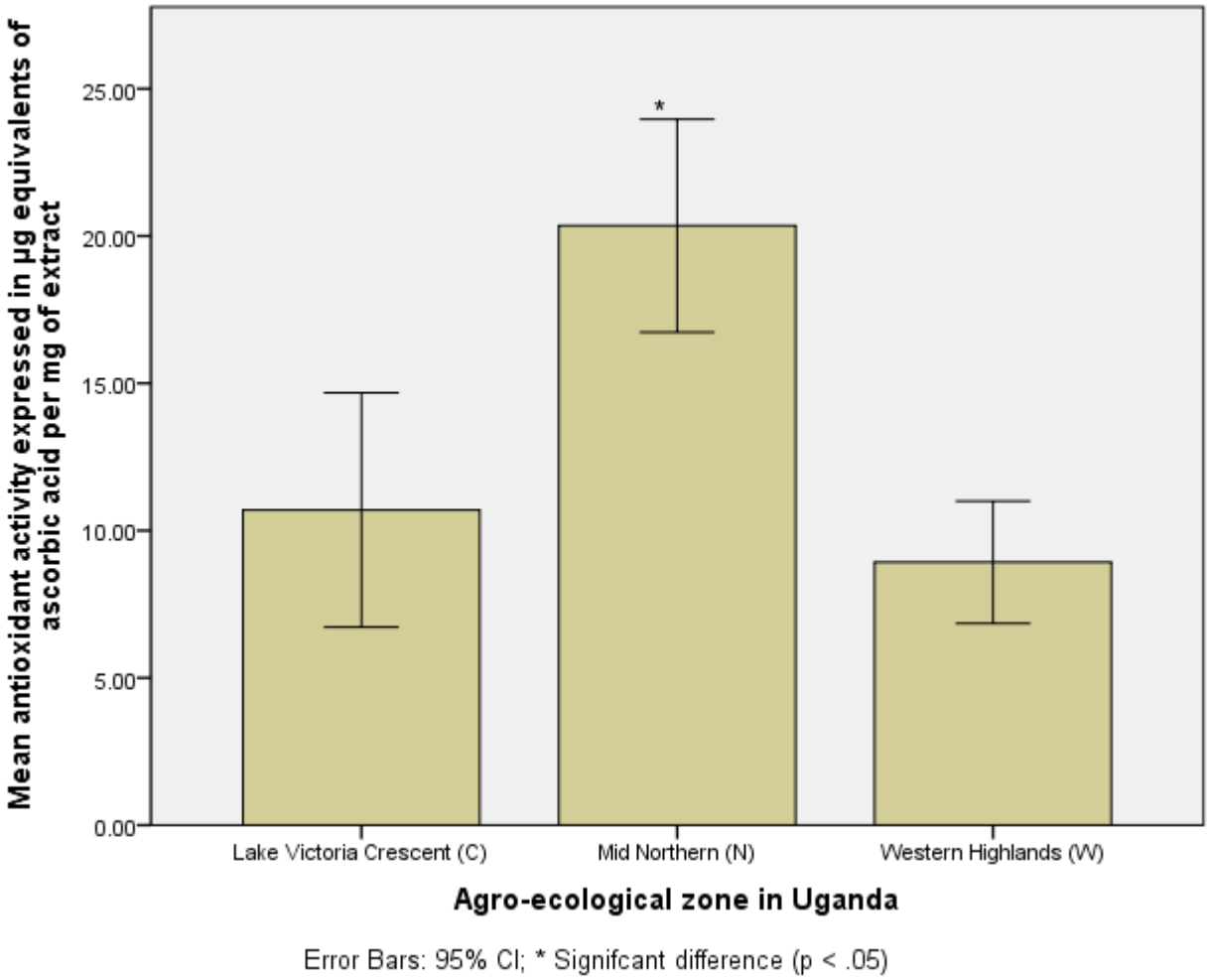


**Figure 3.** Variation of antioxidant activities between propolis samples from different agro-ecological zones. Extracts from mid-northern samples exhibited a statistically significantly higher antioxidant activity compared to those from Lake Victoria crescent or western highlands as determined spectrophotometrically based on DCPIP reduction at a $\lambda_{max}$ of 605 nm.

Katircioglu and Mercan (2006), on the other hand, reported that propolis from Turkey was active against Gram-negative bacteria.

Auamcharoen and Phankaew, (2016), in a study of antibacterial activity and phenolic content of propolis from four different regions of Thailand, concluded that the antibacterial activity of the ethanolic extracts of propolis samples varied in relation to the provenance of the samples. This is consistent with the results of our study. The composition of the plant sources foraged by bees is dependent on their geographical locations which in turn affects the biological activity of propolis produced from those localities (Toreti et al., 2013).

## Antioxidant activity

The absorbance of DCPIP was determined to be strongly correlated with the amount of standard antioxidant added. Thus, the antioxidant activities of the propolis extracts were assessed on the basis DCPIP discoloration with the resulting loss of absorbance; the greater the loss of absorbance, the greater the antioxidant activity of an extract. The 4 mg of propolis extracts of each of the samples used in this study produced significant reduction on DCPIP. This is consistent with other studies that have shown that propolis has antioxidant activity (Socha et al., 2015; Lagouri et al., 2014; Ahn et al., 2007; Al Naggar et al., 2016).

Antioxidant activity varied between apicultural sites within the same agro-ecological zone and certainly between agro-ecological zones. These results are consistent with those of previous studies in which there were differences in antioxidant activity of propolis from various geographic regions (Christov et al., 2006; Kumazawa et al., 2004; Hamasaka et al., 2004; Lu et al., 2003; Sulaiman et al., 2011). Hamasaka et al. (2004), in their study of antioxidant activity and constituents of

propolis collected in various areas of Japan, found out that although Minamiakita and Kazuno, Aizuwakamatsu and Futaba are located in the same prefecture or geographical region, the antioxidant activities of ethanolic extracts were different (Hamasaka et al., 2004). The variation in antioxidant activities between the agro-ecological zones in Uganda might be due to differences in climate and vegetation.

There were significant differences within all the three agro-ecological zones, but only the differences between the mid northern agro-ecological zone and the other two zones were significant. The non-significance of differences in antioxidant activity between the Lake Victoria crescent and Western highlands implies that propolis extracts from these two zones are quite similar in antioxidant activity. This similarity could be explained by the fact that the Western highlands and Lake Victoria crescent agro-ecological zones are both within the tropical savannah climate; the mid northern agro-ecological zone on the other hand is within the semi-arid climate (BakamaNume, 2011).

## Conclusions

In this study, we investigated the *in vitro* antioxidant and antibacterial activities of propolis samples from three agro-ecological zones of Uganda. At a concentration of 2 mg/mL, all the propolis samples used in this study had antioxidant activity as measured by the extent of DCPIP discoloration and the resulting decrease in its absorbance at 605 nm wavelength. All of our samples showed antibacterial activities on at least one bacterial species within the range of concentrations used for assay, up to 200 mg/ml. To the best of our knowledge, we have illustrated the diversity of Ugandan propolis for the first time; antioxidant and antibacterial activity of propolis vary with the agro-ecological zones of Uganda. It is also evident that antioxidant and antibacterial activities vary between apicultural sites within the same agro-ecological zones.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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