



SOCIO-ECONOMIC UTILIZATION OF UPAS TREE, *Antiaris toxicaria* Lesch: A CASE STUDY OF MABIRA CENTRAL FOREST RESERVE, UGANDA

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ABSTRACT

Upas tree (*Antiaris toxicaria* Lesch) is a monoecious and medicinal tree species belonging to the Moraceae family. It is mainly used as timber for construction (plywood) such as beam-making, and treatment of various human diseases. It is used to treat tuberculosis, cancer, blood pressure, cough, ulcers, hepatitis, yellow fever, weakness in pregnancy, headache, wounds, stomach complications (parasitic worms), severe stomach pain during menstrual periods, etc. Upas tree (*Antiaris toxicaria* Lesch) of Mabira CFR is found with poor seedling regenerative capacity due to a very small number to no seedlings. This is attributed to anthropogenic disturbance factors among others. The aim of this research study was to determine the socio-economic factors associated with the utilization of Upas tree (*Antiaris toxicaria* Lesch) in Wakisi and Najjembe Divisions in order to clearly understand more about the local tree species use for proper future management policy and planning, as well as sustainable livelihoods improvement. The study employed a Cross-Sectional Research Design (CSR). Semi-structured questionnaire and participant observations were used during data collection. Purposive selection of 2 Divisions of Buikwe district, 10 villages from each Division, and a random selection of 410 households was done. IBM SPSS software (version 28.0) was employed, while χ^2 was used for data analysis and determination of the association. A high multipurpose utilization of the tree species was found. Residence duration (p -value: 0.000), Occupation (p -value: 0.002), and household monthly income (p -value: 0.028) were significantly associated with the utilization of the multipurpose tree (all p -values < 5%). Further socio-economic studies are needed for other important medicinal trees of the forest. NFA should put more effort into the management of Mabira CFR through its protection, promotion of the tree's domestication, and restoration of the forest, particularly focusing on the conservation of important tree species particularly the *A. toxicaria* Lesch, and the whole forest reserve in general.

Keywords: Upas tree (*Antiaris toxicaria* Lesch), Socio-economic Utilization, Mabira CFR



INTRODUCTION

Upas tree (*Antiaris toxicaria* Lesch) is a monoecious and medicinal tree species belonging to the Moraceae family. It flowers from October to June according to the ecological range of different countries. It can grow up to 25-40 m or more in height with a large trunk and within 20 years. It has a good self-pruning ability. The fruits of the tree are hairy and either orange or dark red, while its sap contains poisonous chemicals. It has broad, recalcitrant seeds of approximately 10 mm in length. Desiccation affects the germinability of the tree seeds (Bai et al., 2011). *A. toxicaria* Lesch is a tree with oblong, oval, obtuse or acute cordate leaves which are hairy with teeth-like extensions on both sides when young (Porchselvi & Muthulakshmi, 2018).

Upas (*Antiaris toxicaria* Lesch) is a fast-growing tall tree. Its fruits are mainly dispersed by birds, bats, monkeys, and antelopes. During its season, *Antiaris toxicaria* Lesch produces a big number of seeds that are easily collected from the ground. However, they lose their viability fast (Orwa et al., 2009). Upas tree (*Antiaris toxicaria* Lesch) is generally distributed in various habitats from wet evergreen forest to dry wooded grassland. Though it is evaluated by the International Union for the Conservation of the Nature (IUCN) Red List as Least Concern, its population size is declining in some areas due to over-exploitation and loss or degradation of habitats (Ugwoke et al., 2017). *A. toxicaria* Lesch is an endemic tree species to some areas of the World such as Kalimantan (Indonesia) and some tropical countries of the Asian continent. The tree is used in traditional medicine and the phytochemical analysis of its latex confirmed the presence of some active chemicals, such as some types of Antiarin (cardenolide /toxicarioside), and many more active chemical compounds (Subiono et al., 2017).

Qualitative phytochemical studies presented the existence of some secondary metabolites including alkaloids, phenols, glycosides, anthraquinone, protein, amino acids, flavonoids, phytosterols, and saponins which were reported to cure different ailments (Kemila & Krishnaveni, 2016; Umdale et al., 2020). Medicinally, Upas tree leaves, seeds, and bark are used as an astringent, febrifuge and seeds are also used as anti-dysenteric. The bark is used as an anodyne, and vermifuge, to treat hepatitis and mental illnesses (Ugwoke et al., 2017). In some countries, the tree is used to treat cancer due to its active chemicals. In some parts of India such as Kerala, the bark of the tree is important in making a type of bark cloth called Maravuri (Umdale et al., 2020). Scientific studies revealed that Upas tree (*A. toxicaria* Lesch) can be used to treat Tuberculosis in East Africa (Obakiro et al., 2020). In Uganda, Upas tree (*Antiaris toxicaria* Lesch) is known to treat headache and weakness in pregnancy (Tumuhe et al., 2018). In various countries, the tree is known for its medicine, plywood, and veneer. However, despite the importance, wide distribution, and dominance of the Upas tree (*Antiaris toxicaria* Lesch) in some countries, over-exploitation together with environmental challenges have been decreasing its population size, whereby only a single individual tree was recorded in some parts of the world. For example, in some parts of India, the extinction of the tree species was recorded (Umdale et al., 2020).



In sub-Saharan Africa, the use of medicinal plants is associated with some socio-economic factors such as educational status, age, marital status, and wealth status among others. In the study conducted in Katikekile Sub-county (Moroto district, Uganda), the socio-economic utilization of medicinal plants was found higher among older people, married people and people living far from modern health facilities. The study revealed that a high socio-economic utilization of medicinal plants has a negative impact on their health (Logiel et al., 2021).

Anthropogenic factors have been putting important medicinal plants at the risk of extinction through different ways such as road construction, grazing, human settlement, and agricultural encroachment. Therefore, a combination of disturbance factors affects vegetative reproduction of plants, especially medicinal woody plants (Chokheli et al., 2020).

In Uganda, Central Forest Reserves (CFRs) particularly Mabira CFR, are homes of medicinal plants (i.e. Trees) to the populations living around them. These plants are mostly collected by herbalists involved in their saleable treatments and they gather them for daily domestic utilization, whereby harvesting consists of gathering leaves, roots, flowers, bark, fruits and seeds according to the plant parts of interest to the collectors. Unsustainable harvesting of medicinal plant parts is a threat to the regeneration of some medicinal plants (Ministry of Water and Environment, 2020).

Upas tree (*Antiaris toxicaria* Lesch) of Mabira CFR was found with poor seedling regenerative capacity due to a very small number to no seedlings (Tumuhe et al., 2018). This was attributed to anthropogenic disturbances among others. Due to its high value, *Antiaris toxicaria* Lesch is under pressure of high utilization by the population living within its proximity such as those living near Mabira CFR. There is a limited information on socio-economic utilization of this tree species. The aim of the study was to determine the socio-economic factors associated with the utilization of Upas tree (*Antiaris toxicaria* Lesch) in Wakisi and Najjembe Divisions in order to clearly understand more about the local tree species use for a proper future management policy and planning, as well as sustainable livelihoods improvement.

MATERIALS AND METHODS

Study area description

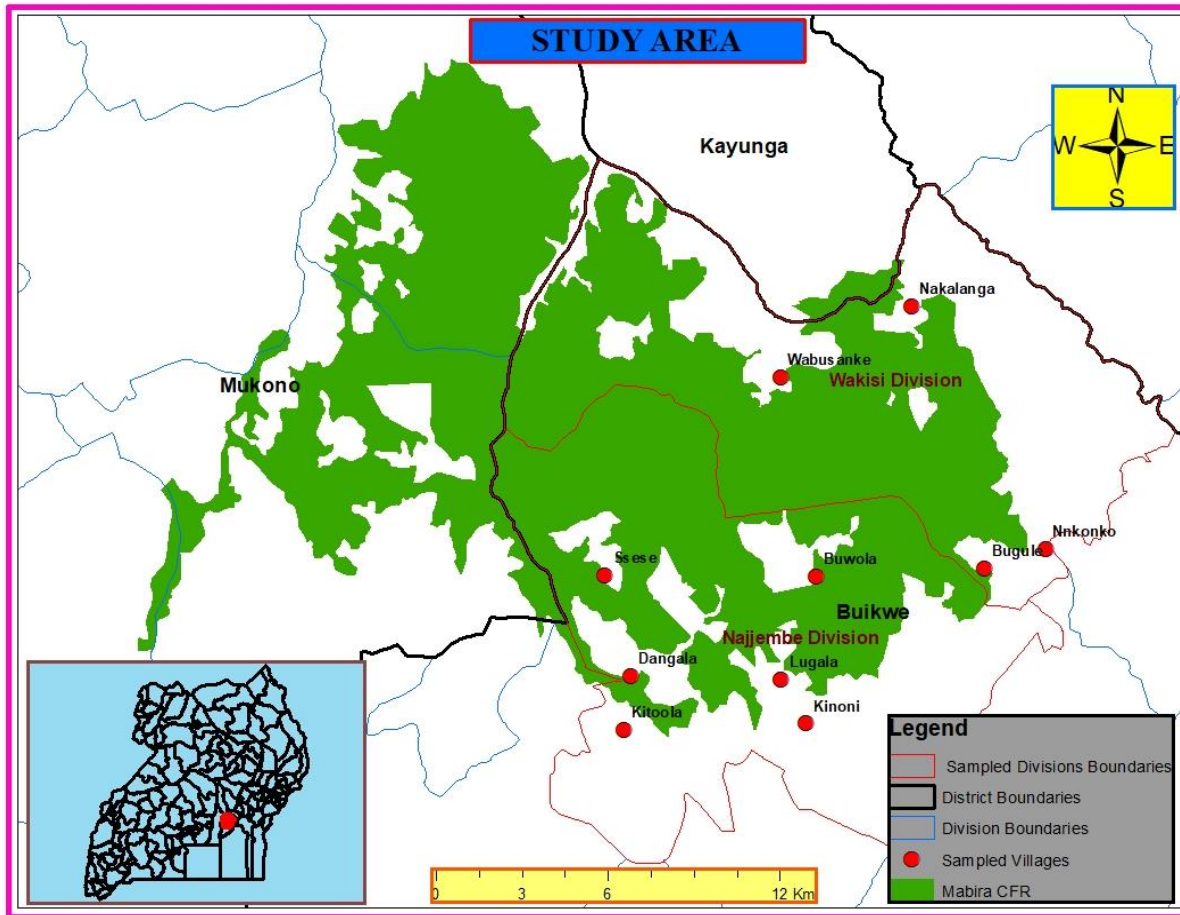


Figure: 1.Map of the study area and its location in Uganda

Mabira CFR covers an area of 29,974 Hectares. It is located in Mukono, Buikwe and Kayunga districts, Counties such as Buikwe, Nakifuma, Mukono and Ntenjeru and Sub-counties such as Wakisi, Nagojje, Najjembe, Kimenyedde, Nama and Kangulumira of Kayunga district. Geographically, Mabira CFR is located between latitude $00^{\circ} 22'$ and $00^{\circ} 35'$ N and between $30^{\circ} 56'$ and $33^{\circ} 02'$ East. The management and control of Mabira CFR is vested in the National Forestry Authority (NFA) under the National Forestry and Tree Planting Act, 2003 (Ministry of Water and Environment, 2020).

Data collection and research design



Buikwe district holds a total of 97,833 households. Najjembe sub-county (Division) has a total of 8,007 households, while Wakisi sub-county (Division) has 9,256 households (UBOS, 2014). From the total households' number (17,263) of the two sub-counties, YAMANE's formula ($n = \frac{N}{1+N(e)^2}$) was used to estimate the sample size; where n : sample size; N : population size

and e : margin error/level of significance (5%) from the confidence level of 95%. A total of 391 households (respondents) was estimated. In addition, five percent (5%) of this number was added to cater for non-response due to unexpected reasons such as very old age (unable to talk well), etc. Therefore, a total of 410 respondents (household heads) was randomly selected from the Divisions.

The present study employed a cross-sectional research design. It was carried out in Mabira CFR and communities living around the forest reserve. It used only primary data, while a Semi-structured questionnaire with 4 sections was used. Section 1 consisted of the identification and characteristics of the respondents per households, while the remaining sections consisted of different questions (open and/or close-ended questions) according to the research objective. From a total of 410 respondents (each from the selected households) for both sub counties, 246 respondents were randomly selected from Najjembe Division while 164 were selected from Wakisi division. From Najjembe Division, six (6) villages were purposively selected while four (4) villages were purposively selected from Wakisi Division. The purposive selection of villages was based on their neighborhood to Mabira CFR. Forty-one (41) households (respondents) were randomly selected from each village. In addition, participant observations were made to support and complement the survey.

Statistical analysis and data presentation

The gathered data were scrutinized (assessed several times to ensure that there was no missing information, especially made during recording) to avoid errors. This was done immediately after data collection and missing information was removed from the sample in order to avoid bias. The collected data were recorded in tables and retrieved for their processing and analysis.

The collected data were analyzed by employing the Statistical Package for Social Sciences (IBM SPSS, version 28.0). Inferential analysis was used to show the association between the utilization of Upas tree (*Antiaris toxicaria* Lesch) and socio-economic factors, whereby Chi-Square (χ^2) was used to determine the significance in dependency of socio-economic factors on the utilization of the Upas tree in the communities around Mabira CFR. Type I and Type II errors were controlled by using confidence level of 95% and significance level of 5%. The results were presented in chart form. Chi-square analysis was used as exemplified by Mhuji et al.(2018).

RESULTS AND DISCUSSION

Collection and local utilization of Upas tree

This section presents the descriptive statistics on the collection and utilization of *Antiaris toxicaria* Lesch. With a total number of 410 respondents from which the survey was conducted, only 309 respondents answered to the question.

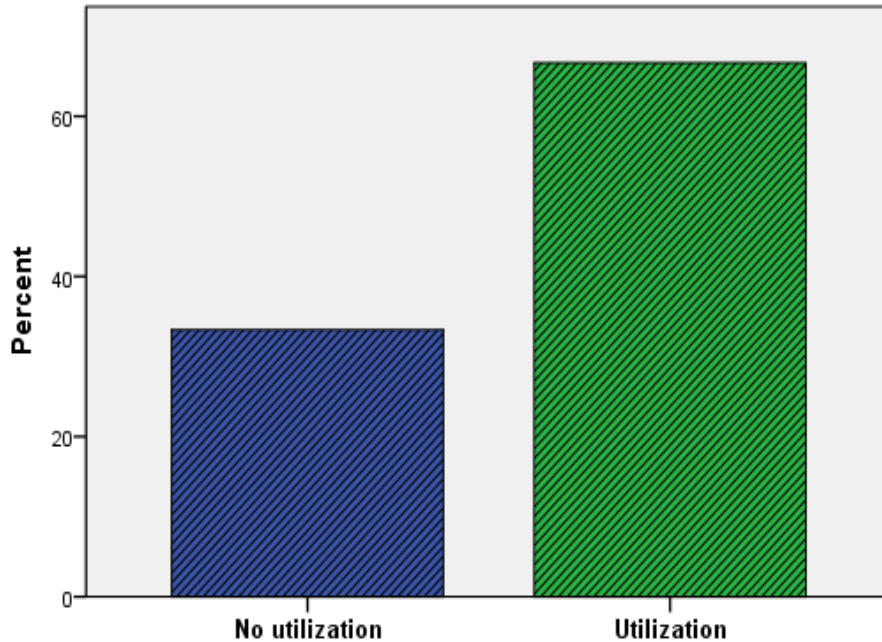


Figure: 2.Collection and utilization of *A.toxicaria* Lesch

According to the results of the present study (Fig.2), the local utilization of the Upas tree (*Antiaris toxicaria* Lesch) was found to be at 66.7% in the study area. These findings confirm those of Logiel et al., (2021) about the utilization of medicinal plants in Katikekile Sub county, Moroto District (Uganda), where the utilization of the plants was found to be 68 %. In both the studies, the utilization is relatively high. The findings of this study are not consistent with those of Mhuji et al., (2018) who found a lower utilization of medicinal plants of less than 40%. The findings are not also consistent with those of Andriamparany et al., (2014) in Madagascar with an overall high collection and utilization of medicinal plants of 82%. According to the results, participant observation, and conversation with the local people, this utilization is high for such a single tree species of the forest. This is not only shown by the above proportion (Fig.2), but also by trees cuts in the forest, a very small number of young trees in the forest, timbers from the forest's tree found in some families, houses constructed with timbers from the tree resource, and a high loss of the trees in the local area. This illustrates a pressure that local people have been putting on the forest for the tree resource, probably due to a high level of unemployment in the study area.



Photo 1. Collection and utilization of *Antiaris toxicaria* Lesch from Mabira CFR

The high utilization of the tree species should be minimized through more local job creation, planting the tree species in both the forest and local people's gardens as the tree can coexist with other crops, and incorporation of local people into the management of the forest.

Local utilization (importance) of Upas tree

With 66.7% of collection and local utilization, eight (8) local uses of *Antiaris toxicaria* Lesch were identified (Fig 3). The tree species is used to treat some human (23.6%) and livestock diseases (1.4%), to make clothes: 4.2% (bark cloth: Embugo), as timber (soft wood) in construction: 42.1% (especially in beam-making), for both construction and commercialization (2.8%), as fertilizer (2.3%), sold for income generation (commercialization: 18.5%), as well as firewood (0.9%). Among the local uses found, Upas tree is mainly used as timber for construction (42.1%), and treatment of some human diseases (23.6%). However, according to the respondents' information, more than fifteen diseases were reported to be treated by Upas tree (*A. toxicaria* Lesch). The reported diseases were headache, mental illness, weakness in pregnancy, yellow fever, skin diseases (i.e. rash), wounds, cough, ulcers, Severe stomach pain during female menstrual cycle (Ekigalanga), stomach complication (i.e. Parasitic worms), diabetes, blood pressure, skin rash occurring amongst babies (Enoga), Ania, as well as treating poultry diseases. In addition, the tree species was used to treat Covid-19 symptoms (i.e. cough) during the covid-19 pandemic as reported by some households (8.6% of 233 respondents who answered to the question). The most tree parts used in disease treatment are stem bark and leaves, respectively. Descriptive statistics was used to find the above percentages at the Standard Deviation (SD) of 3.328. The Mean, Median, and Mode were found to be 5.43, 8.00 and 8 respectively. Moreover, the tree species was reported to contribute to some household's livelihood improvement through income generation from sold timbers, sold medicine, sold bark-cloth (Embugo), medicine in households (homes), firewood, and construction at home.

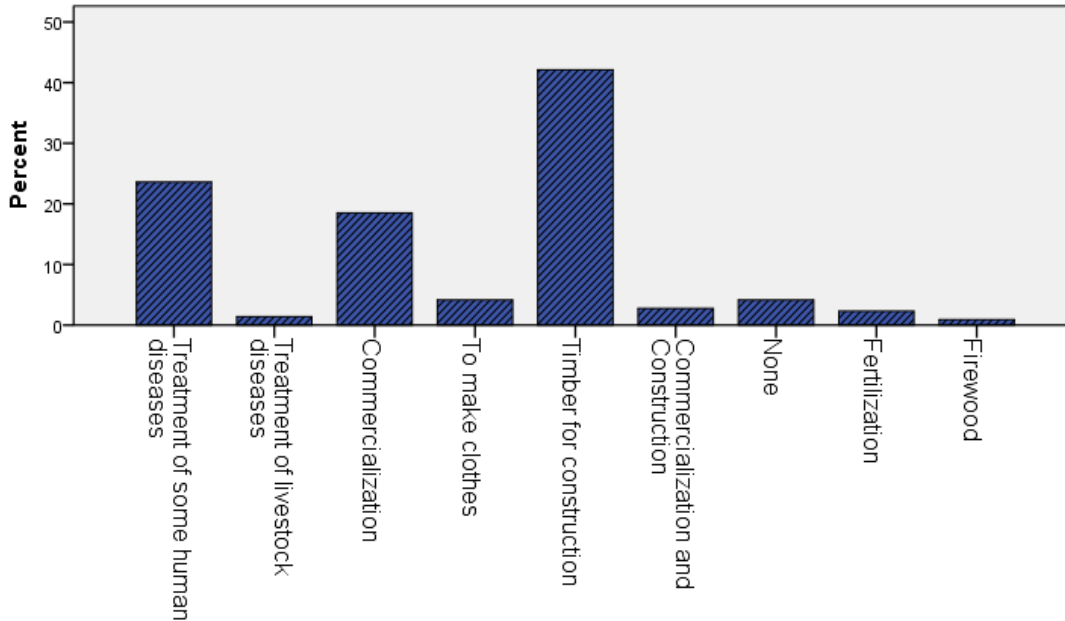


Figure: 3. Local utilization (importance) of *A. toxicaria* Lesch

Upas tree is used to treat is multipurpose plant as it is utilized for various reasons, mainly in construction and disease treatment. The study identified numerous diseases that are treated by the tree resource. Treatment of weakness in pregnancy and headache was also documented by Tumuhe et al., (2018), while the treatment of wound, parasitic worms, and mental illnesses was identified by (Ugwoke et al., 2017). Bark cloth from *Antiaris toxicaria* Lesch was also identified in India (Umdale et al., 2020).

Medicinal importance of different tree species was also reported by Andriamparany et al., (2014). In the study of Abdourhamane et al., (2015), it was found that woody plants are used as wood energy, human disease treatment, livestock disease treatment, fodder, human food, woody services and handcraft. Therefore, being two different research studies on different trees, this study found similar and different uses of woody plants compared to the study of Abdourhamane et al., (2015). However, while some other medicinal trees such as *Prunus africana* Miller were found to be more valuable as medicine than their timbers (Galabuzi et al., 2021), the findings of this new study demonstrate that Upas tree (*Antiaris toxicaria* Lesch) is more valuable as timber than medicinal source, probably due to a lack of knowledge on its medicinal importance found in various households. This study also clears the doubt that *A. toxicaria* Lesch is not a source of bark cloth as its bark is used to make the cloth (Embugo) which covers a dead person before being buried, probably making its bark to be the most used tree part. According to the responses from the study area, in Buganda region, dead people have to be buried in bark cloth (Embugo). This is an additional fact showing how the tree species is at high risk of utilization or it is endangered, though



it is evaluated by the IUCN Red List as Least Concern (LC). Therefore, there is a need to conserve this tree species.

Association between age and local utilization of Upas tree

The results were obtained by using inferential statistics through Chi-Square test statistics. It is shown that the utilization of Upas tree was high amongst middle age (31-43 and 44-56) while the highest utilization was found to be within the age range of 31-43 (Fig 4). However, the overall utilization of Upas tree with respect to age was found to be insignificant (p -value: 0.065, greater than significance level of 5%). Therefore, there is no association between the age of respondents and the utilization of Upas tree.

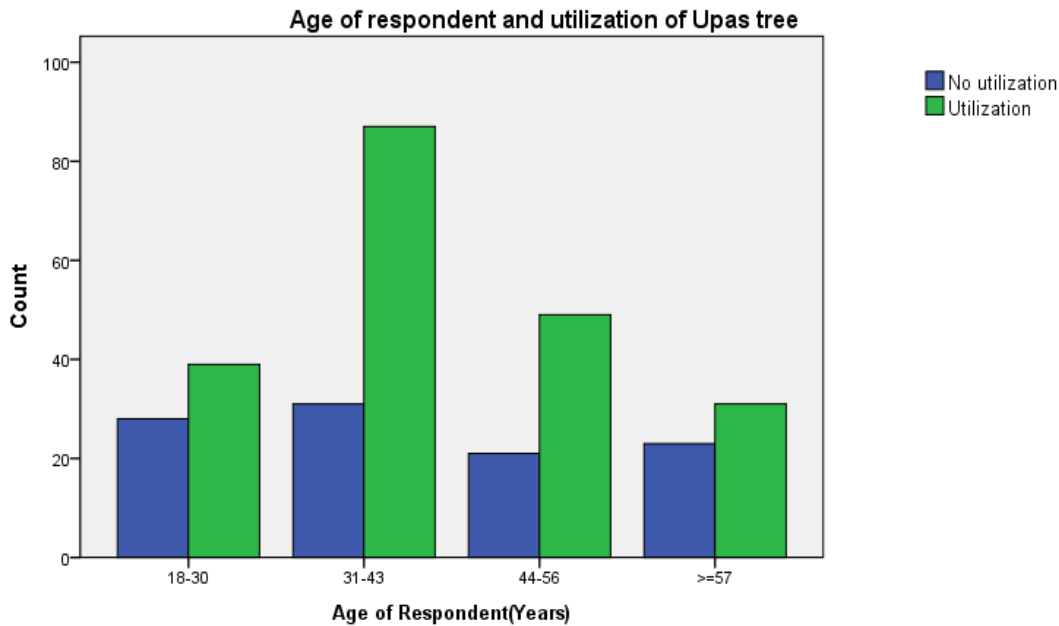


Figure: 4. Association between the age of respondents and the utilization of *A.toxicaria* Lesch

The overall utilization of Upas tree with respect to age was not found significant. Consequently, there is no association between the age of respondents and the utilization of Upas tree. The data provides a new insight that age distribution does not influence the utilization of the tree resource. The findings of this study are not consistent with the findings of Mhuji et al., (2018) and Logiel et al., (2021) which found the utilization of medicinal plants to be higher among older age. However, the findings are consistent with those of Bari et al., (2017). Therefore, the results of this study confirm the research hypothesis that there is no association between age and the utilization of Upas tree.

Association between the level of education and utilization of Upas tree

The results were produced to test whether there is an association between education and the utilization, while only a total number of 309 household heads responded.



The findings were obtained by using inferential statistics through Chi-Square test statistics. They show that the utilization of Upas tree is higher amongst the low level of education (No formal education, Primary education and secondary education respectively). The highest utilization was found to be amongst the lowest level of education (Fig 5). The utilization increases with the decrease in education level. However, according to the results, there is no association between the level of education and the utilization of Upas tree (p -value: 0.615 greater than significance level of 5%). This supports the research hypothesis.

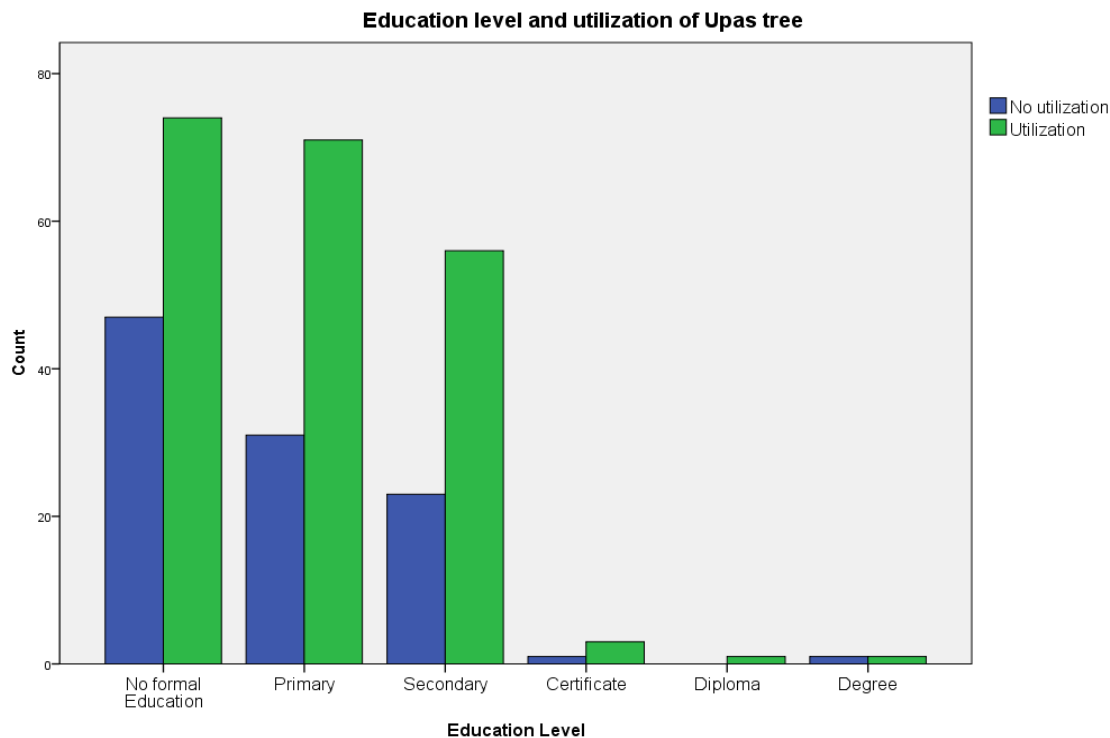


Figure: 5. Association between the level of education and the utilization of *A. toxicaria* Lesch

The highest utilization was found to be amongst the lowest level of education (no formal education). This is probably due to a high unemployment level amongst people with no formal education and few income sources in the study area as it is a rural area. The data provides new evidence that education level does not influence the utilization of Upas tree. The results from this study are consistent with the results of the study conducted by Mhuji et al., (2018) whereby an increase in education level was associated with a decrease in the utilization of medicinal plants. However, the findings of this study contrast the findings of Bari et al., (2017) and Logiel et al., (2021) whereby, a higher education level was significantly associated with the utilization and contribution of medicinal plants. Therefore, the present results confirm the research hypothesis that there is no association between education level and the utilization of Upas tree.

Association between Residence duration and utilization of Upas tree



This section reveals the findings about whether there is an association between the residence duration of respondents and the utilization of Upas tree. Only 309 responses were obtained. By using inferential statistics with Chi-Square, the utilization of Upas tree was found to be the highest amongst people who resided in the study area for a long time (i.e. from 18 years and above). See (Fig 6). The p -value was found to be statistically significant (sign: 0.000, much lower than the significance level of 5%). Therefore, there is a significant association between residence duration and the utilization of Upas tree, hence no consistence with the null research hypothesis.

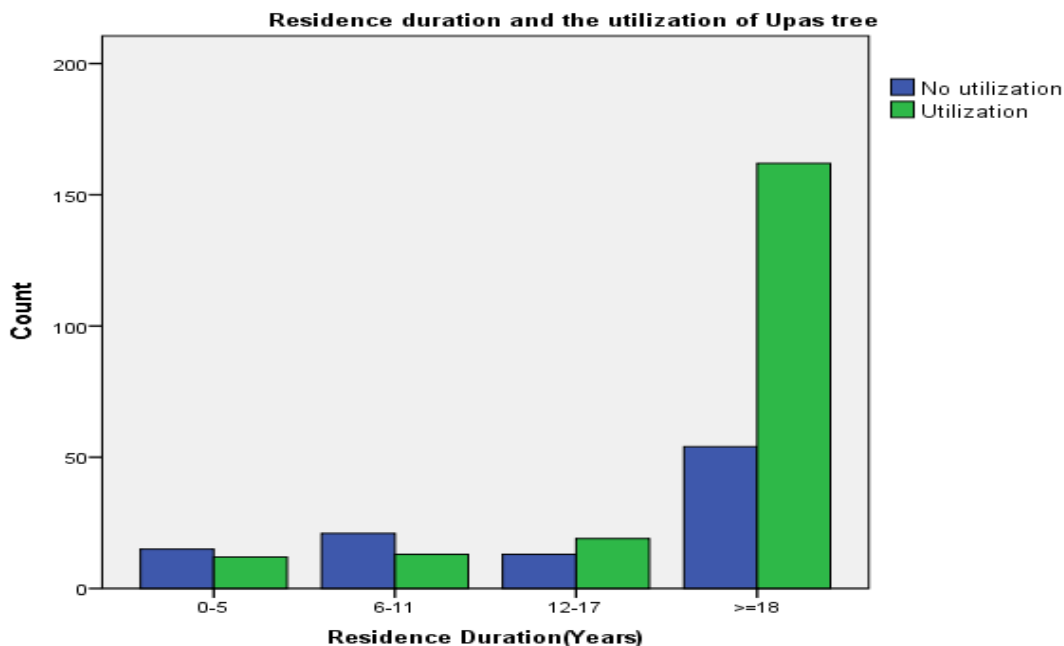


Figure: 6. Association between residence duration and the utilization of *A. toxicaria* Lesch

The results show a significant association between residence duration and the utilization of Upas tree. This means that the utilization of Upas tree is influenced by residence duration. This provides an insight into the importance of using indigenous knowledge in medicinal plant species utilization around Mabira forest. People who resided in an area for a long period of time, have acquired more knowledge of trees' use as they got enough time to explore the area.

The findings of this study are consistent with the findings of Mhuji et al., (2018) which show an association between residence duration and the utilization of medicinal plants whereby a long period residence time in village was related to an increase in collection and utilization of medicinal plants. The findings of this study reject the research hypothesis.

Association between household size and the utilization of Upas tree

The next graph presents the findings on the association between household size and the utilization of Upas tree. Here, responses obtained were only 309. The results show that the utilization of the tree resource increases with a decrease in household size (Fig 7). The highest utilization was found amongst the smallest size class (From 1 to 4 family members). Chi-square test (inferential



statistics) results showed a p -value of 0.371 which is greater than the significance level of 5%. Therefore, there is no association between household size and the utilization of Upas tree, which supports the null hypothesis.

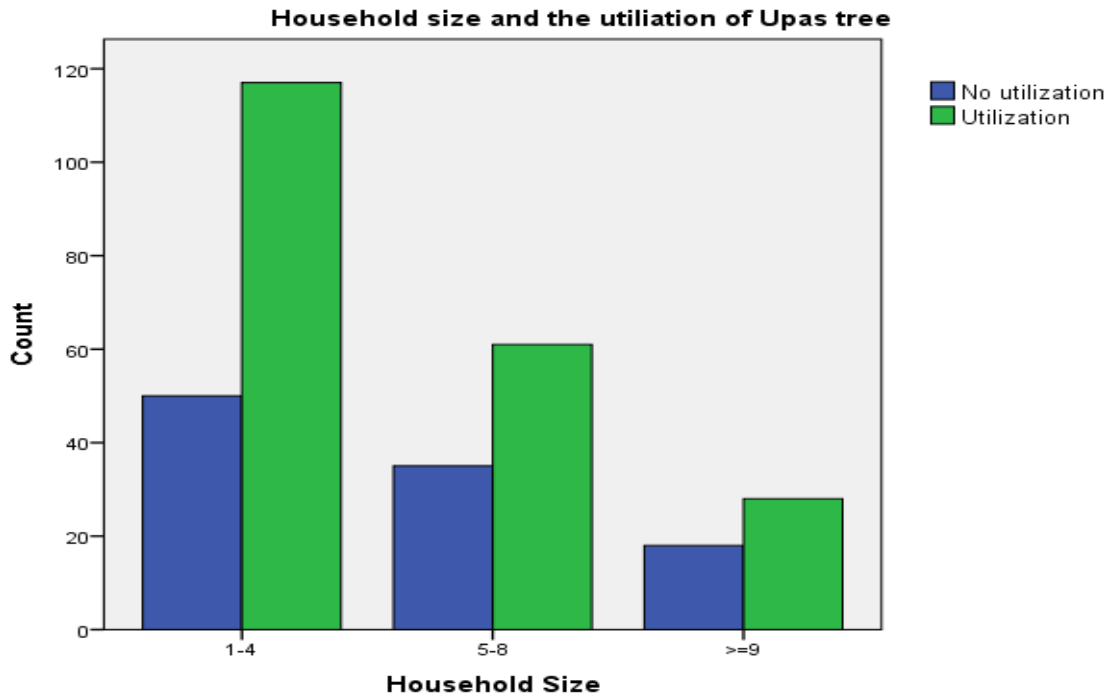


Figure: 7. Association between household size and the utilization of *A. toxicaria* Lesch

The utilization of Upas increases with a decrease in household size and the highest utilization is amongst the smallest household size class. This is probably due to higher energy and more time of few household members enabling them to work especially in agriculture, compared to many household members. According to the results found, there is no association between household size and the utilization of Upas tree. The data provide an evidence that household size does not influence the utilization of Upas tree. The study's findings are not consistent with the findings of Andriamparany et al., (2014) and Bari et al., (2017) from which family size was significantly associated with the utilization and contribution of medicinal plants with effect on medicinal woody plants and influence on socio-economic conditions. Therefore, the results confirm the research hypothesis.

Association between the occupation of respondents and the utilization of Upas tree

Responses were collected from a total number of only 309 respondents who responded. The utilization of Upas tree is very high amongst the occupation class of farmers (Fig 8). The Chi-square test results show a p -value of 0.002(sign). This value shows a statistical significance in the results as it is far below the significance level of 5%. Therefore, there is a significant association between the occupation of respondents and the utilization of Upas tree. This rejects the null hypothesis.

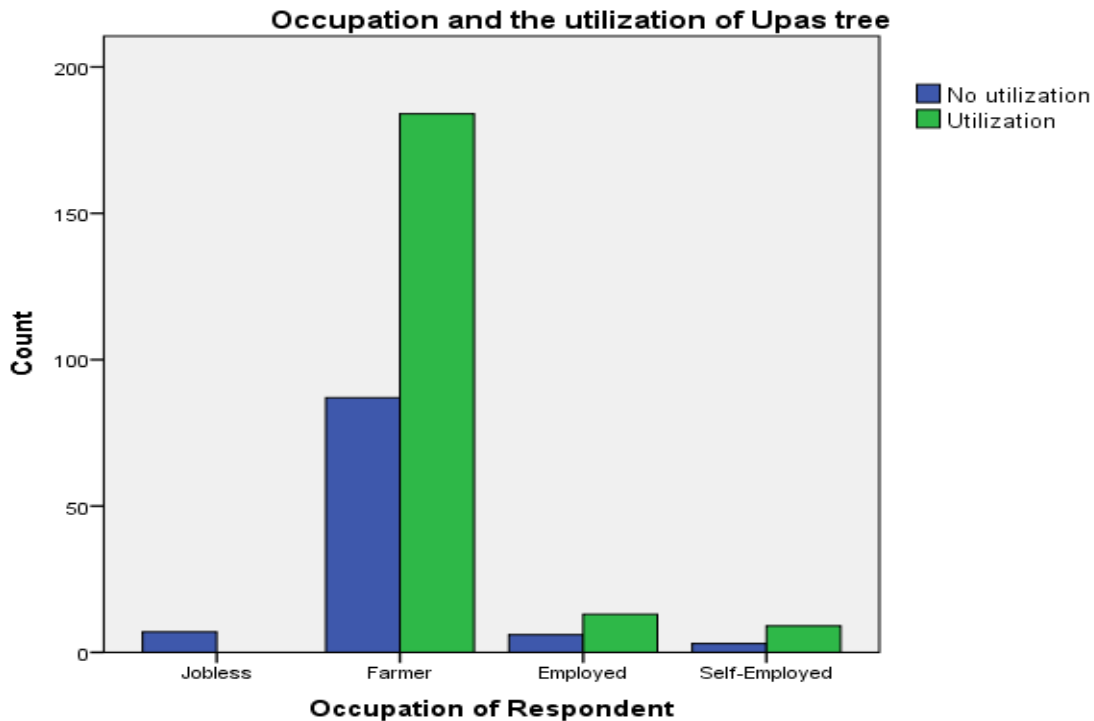


Figure: 8. Association between the occupation of respondents and the utilization of Upas tree

From the results above (Fig: 8), the utilization of Upas tree was found the highest among the occupational class of farmers. This is due to the fact that farmers spend most of their time gardening around the forest. They have tried and tested the species' plant parts for its various uses. However, the results show a significant association between the occupation of respondents and the utilization of the tree resource. The data provide a new evidence that the occupational class significantly influences the utilization of the tree resource. The findings of this study are consistent with the findings of a study conducted in Tanzania from which farmers were the main collectors and users of medicinal plants (Mhuji et al., 2018).

Association between respondents' family monthly income and the utilization of Upas tree

Out of 410 expected responses, only 309 were collected. The results show a high utilization of Upas tree among low income families (household class earning 1-100,000 and 101,000-200,000 Uganda shillings per month). The highest utilization is amongst households earning 101,000 to 200,000 Uganda shillings per month (Fig 9). From Chi-square results, the p -value is 0.028, which is less than the level of significance of 5%. Therefore, according to the results, there is a significant association between household monthly income and the utilization of Upas tree. Therefore, the assumption of the null hypothesis is rejected.

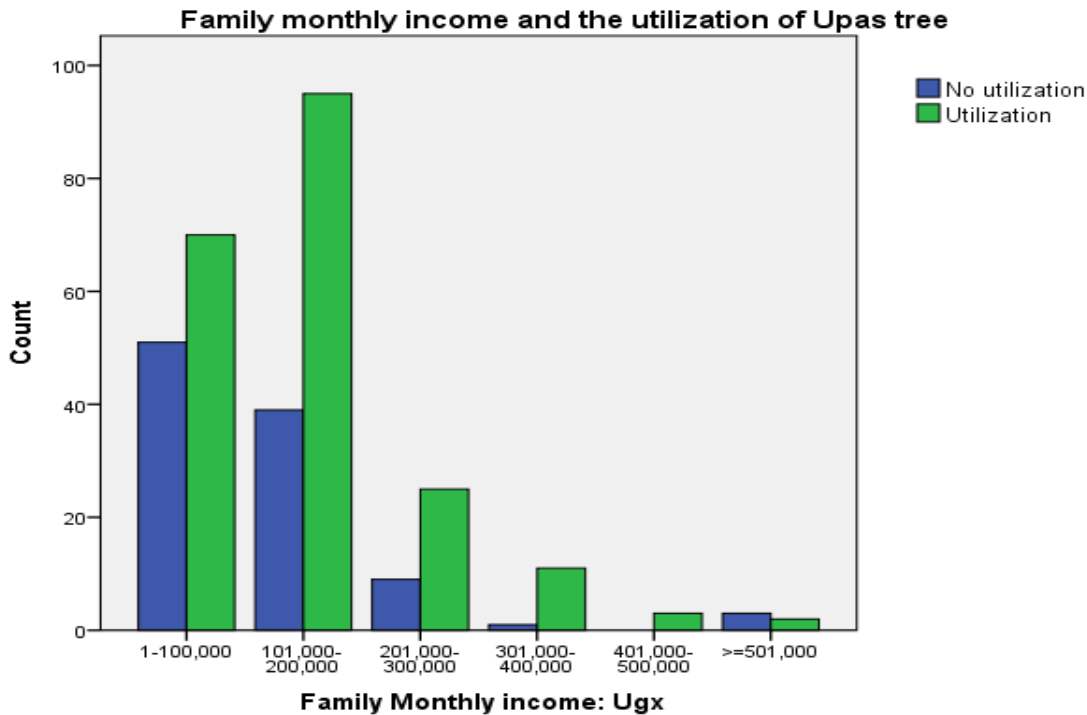


Figure: 9. Association between respondent's household monthly income and the utilization of *A. toxicaria* Lesch

The results of this study presented a high utilization of Upas tree amongst low income households. The tree resource utilization increases with a decrease in wealth and vice-versa. The highest utilization is among low income households. This is due to the fact that medical care from modern health facilities is relatively expensive in terms of monetary value, so, the community with low income resorts to species utilization to save on the cost of treatment. In addition, poor households intend to use forest resources as an alternative source of income as their income sources are limited. An association was found between household monthly income and the utilization of Upas tree. The presented data provide a new evidence that the utilization of Upas tree is affected by household monthly income. However, the findings of this study conform to those reported by Mhuji et al., (2018) who observed an association between the use of wild medicinal plants and household wealth. The same study concluded that wild medicinal plants utilization decreases as household income increases.

The main challenge encountered during this socio-economic survey was the change in the number of responses according to the different socio-economic factors due to non-responses which might affect the reliability of the results. The total number of responses collected is too small to be generalized for the whole communities around the forest reserve. These findings should not be generalized for all the communities around Mabira CFR since the data were collected from only one district (Buikwe), while the forest is held by 3 districts. Therefore, further research studies



should take into account the remaining two districts (Kayunga and Mukono). The percentage number to be added on the sample size to cater for non-responses should be increased.

CONCLUSION AND RECOMMENDATION

The study showed a high socio-economic utilization of *Antiaris toxicaria* Lesch within the study area and the tree was found to be useful for various purposes. Residence duration, occupation of household, and household monthly income were significantly associated with the utilization of Upas tree (*Antiaris toxicaria* Lesch), hence influencing such a utilization. Other socio-economic factors were not significant. NFA should put more effort into the management of Mabira CFR through its protection, promoting tree domestication, and forest restoration. It should focus on important tree species such as *A. toxicaria* Lesch (Upas tree) among others. Sensitization about the importance of the forest, more job creation, and the promotion of education in the study area should also minimize the high utilization of some important trees from the forest. Future research is needed to study the socio-economic utilization of other important medicinal tree species of the forest in order to assess their local use as the forest is getting thinner.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *Commelina diffusa* SHOOT EXTRACT AGAINST *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

Background. Bacterial infections remain the leading health complications not only in Uganda but also in abundant in Africa at large. Although recent developments and trials of various antibiotics are being used in the medical field, the need for very active antibiotic drugs is urgently needed to mitigate the rapid increase of antibiotic resistance (AMR) to major pathogen bacteria. In 2022, 4.95 million deaths were recorded to be associated with bacterial infections which resulted in greater than 6 billion dollars in expenditure in health care (Murray *et al.* 2022). 65% - 80% of people in various countries of the world use medicinal plants as remedies in different parts of the world. This study was carried out to document scientific evidence about the phytochemical composition of a wild plant *Commelina diffusa* and its antibacterial activity of the crude methanolic extract against *Escherichia coli* ATTC 25922 and *Staphylococcus aureus* ATCC 29923.

Methods. An experimental study was conducted between March 2023 and May 2023 on a crude extract of *Commelina diffusa* in the Chemistry and Biology laboratories of Kyambogo University. 3000g of a fresh plant was collected from a swamp area of Banda in Nakawa West division, air-dried and crushed using an electronic crusher in the chemistry laboratory. 150g of the powder was macerated in 750ml of 99.5% concentrated methanol. Phytochemical screening was done using common chemical methods whereas the antibacterial effect was determined by well diffusion method using agar and a 2-fold serial dilution method. Statistical data collected from the experimental determinations was analyzed using Graph Pad Prism version 8 software. Results for anti-bacterial activity (mean zones of inhibition and standard deviation) were analyzed using one-way ANOVA and Turkey's multiple comparison test.

Results. A total of 10 key phytochemicals were screened among which 7 phytochemicals were confirmed present (alkaloid salts, tannins, phenols, coumarins, anthracenocides, saponins steroid glycosides). Phenolic compounds showed an intense concentration whereas the rest 3 (anthocyanocides, reducing sugars and flavonoids) were confirmed absent. *Commelina diffusa* shoot extract has a high anti-bacterial effect on *Escherichia coli* cells with an inhibition zone of 20.5mm and on *Staphylococcus aureus* cells with an inhibition zone of 18.5mm all of which is higher than standard drug tetracycline with inhibition zones of 16.0mm and 14.5mm respectively.



Antibacterial screening results were considered significant for both *Escherichia coli* and *Staphylococcus aureus* at confidence levels of $p = 0.0016 < 0.05$ and $p = 0.0001 < 0.05$ respectively.

Conclusion. This study showed a high significant level between the Phyto-constituents and antibacterial potential of the methanolic extract, supporting its traditional use.

Key words. Antibacterial activity, antibacterial resistance, medicinal plants, traditional medicine, pathogens, serial dilution, inhibitory concentration, bactericidal concentration, zone of inhibition.

INTRODUCTION

Globally, there is an urgent need to reduce the burden of death due to bacterial infection. 7.7 million of deaths accounting for 13.6 % global death associated with drug-resistant infections due to resistant bacterial pathogens, including but not limited to *S. aureus*, *Streptococcus pneumoniae*, *Enterococcus*, *Mycobacterium tuberculosis*, *E. coli*, among others (Criss Dall *et al* 2022). A study that investigated the bacteriuria rate among women attending Mulago Hospital showed *E. coli* (57.5%) and *S. aureus* (22.5%) as key causative organisms among the culture isolates (Mwaka *et al*. 2011). *Escherichia coli* and *Staphylococcus ssp*, are found to be the most predominant uropathogenic isolates of bacterial infections, especially bacterial urinary tract infections (UTIs), bacteremia, diarrhea, neonatal meningitis and pneumonia (Odoki *et al*. 2019). This accounts for about 75-90% of the culture laboratory culture isolates (Kasew D, M. & Afework B, *et al* 2022).

Traditional medicine (TM) occupies a great place in the management of diseases because it is readily available, free or cheap, culturally accepted and believed to be effective (Oregaba *et al* 2011) & (Van Andel *et al* 2013). Most traditional medicines are obtained from plants. Many modern allopathic medicines originate from plants, such as antimalarials. For example, *Atropine* is manufactured from *Atropa belladonna*, (Mohamed, Labib, Khadija & Mustapha *et al*. 2012) and *Camptothecin* is an anticancerous drug from *Camptotheca acuminata* (A. Lorence *et al*. 2004).

Commelina diffusa shoot has several local medicinal uses which include treating UTIs and respiratory tract infections (RTIs), swellings, inflammation, diarrhea, hemorrhoids, and conjunctivitis, among others. (Md. Sohel Rana, Afroz & Nasrin Mahmuda *et al* 2019). The plant is also believed to contain antimicrobial, cytotoxic, antioxidant, antifungal, hepatoprotective and CNS depressant properties (Prima, Ahmed, Faruk & Zaira *et al* 2019).

Within China, *Commelina diffusa* shoot is used as a medicinal herb with febrifugal and diuretic effects (Lansdown *et al* 2018), whereas in Nigeria, *Commelina diffusa* leaf decoction is used to treat fever and used as a leaf-infusion eye wash. Pounded leaves mixed with seeds of *Leea guineensis* are topically used to treat buboes and swollen glands, as a rubefacient in rheumatism (*commelina diffusa* burm. f. [family commelinaceae] on Justor n.d.). In Ghana, it is used to treat swellings in the groin, whereas in Uganda mostly in Districts of Nakapiripirit, Mukono and Kanungu, *Commelina diffusa* is used in the treatment of inflammation, burns, Ophthalmia, leprosy, and sore throat, among others (Md. Mizanur Rahman & Rajia Sultan *et al* 2021). Moreover, in some areas of Kabale and Rukungiri districts, it is used to treat ear infections and fungal infections that affect the head region (jstor *et al*. 2018).



However, apart from this traditional richness of knowledge concerning the medicinal values of the plant, its phytochemical composition and antibacterial efficacy are far incomplete. This study was conducted to acquire scientific evidence that can be used develop effective antibacterial medicines by screening the crude plant extract for Phyto-constituents and antibacterial effects against *Escherichia coli* and *Staphylococcus aureus* bacteria.

MATERIAL AND METHODS

This was an experimental study conducted at the Department of Biological Sciences in the Biology and Chemistry laboratories of Kyambogo University, central Uganda.

Sampling procedure

Random sampling technique (RST) was followed. A small sample of fresh sample of *C. diffusa* shoot weighing 3000g was collected from a swampy area by taking samples from random points along the main stream in Kisenyi-Banda, Nakawa division, central Uganda. The sample was washed with tap water, dried at room temperature under the shade and later crushed using an electronic crusher (ZENGHAI electric herb grinder 200w).

Extraction process

An extraction procedure was used for plant samples through cold maceration, already established by *Abdullahi R. Abubakar & Mainul Haque 2020*. The first step was to take 150g of the powder obtained through the electronic crusher during the sampling procedure and macerate it in 750 ml of 99.5% concentrated methanol solvent for 48 hours, with shaking at regular intervals to enhance on the extraction of active components and then filtered using Whatman grade 1 qualitative filter papers into an amber bottle. Finally, a BUCHI Rotavapor R-205 evaporator was employed to recover the solvent. 40.2g of crude extract was obtained.

Stock solution preparation.

3g of the crude extract was measured using an Advanced ML-T Analytical Balance, Mettler Toledo, dissolved in 1ml of dimethyl sulfoxide and the resultant solution used for the antibacterial activity screening test. 1 ml of the crude extract was serially diluted using a 2-fold serial dilution from 1/2 up to 1/64. Dimethyl sulfoxide solution acted as a negative control, whereas 1 ml of tetracycline acted as a positive control.

Determination of Phytochemical composition.

10 key phytochemicals were screened using common phytochemical screening methods and chemicals available in the Chemistry lab. The procedure that was followed is described below.

Alkaloid: To 1ml of crude extract in the test tube was added 1ml of Wagner's reagent drop wise.



Saponin: To 1ml of crude extract, 2ml of distilled water were added followed by vigorous shaking.

Tannins and phenols: About 2ml of the extract were put in a clean dry test tube and 3-4 drops of 3% ferric chloride solution then added.

Reducing compounds (Fehling's test): 2ml of a crude extract were put in a clean test tube and then added 4ml of freshly prepared Fehling's solution (Fehling's one plus Fehling's two in ratio 1:1). The mixture was then heated in a water bath.

Hydrolysis to extract coumarins, anthracenocides, flavonoids, steroid glycosides and anthocyanocides. 25ml of the extract was measured in a round bottom flask followed by addition of 15ml of 10% of hydrochloric acid, then warmed in a water bath for a period of 30 minutes, allowed to cool and transferred to a separating funnel and added 15ml of diethyl ether. The funnel then shaken and allowed to settle separating the two layers. Both the upper (ether) layer and lower (acidic) layer were collected in separate test tubes and used for carrying out the following tests.

Anthracenocides: About 4ml of the ether extract (upper layer) were placed in a clean test tube and evaporated to half (2ml) in a water bath, then 2ml of 25% ammonia added while shaking.

Anthocyanocides: The lower acidic solution (bottom layer) was divided into two equal proportions in two clean test tubes, to the first tube was added 2ml of 25% of ammonia solution and the second was used for reference.

Coumarins: About 2ml of the ether extract (upper layer) were placed in a boiling tube followed by heating to dryness and the resultant was dissolved in 2ml of 10% ammonia solution. A pale green fluorescence was seen under U.V light.

Flavonoids: 2ml of the ether extract were placed in a boiling tube and heated to dryness. The residue was then dissolved in 2ml of 50% methanol by heating. A piece of magnesium was then added, shaken and 1ml of concentrated hydrochloric acid added and then observed under U.V light.

Steroid glycosides (Salkowski's test): About 2ml of the ether extract (upper layer) were added to a boiling tube and then evaporated to dryness in a water bath; to it was added 1ml of chloroform followed by 1ml of 98% concentrated sulphuric acid.

Determination of antimicrobial activity

In-vitro antibacterial activity of the crude extract was assessed against *Staphylococcus aureus* strain (ATCC 29923) and *Escherichia coli* stain (ATCC 25922) following standard agar well diffusion method.

1. Pure isolates of the test microorganisms were cultured on Mueller Hinton agar at 37°C for a period of 24 hours, where 1ml of the test bacteria was spread onto the agar on the Petri-dish/plates for *Escherichia coli* and *Staphylococcus aureus*.



2. 3 wells of 6mm diameter each were dug in the inoculated agar plate using a sterilized borer. 3g of the crude extract was measured on an analytical weighing balance and then dissolved in Dimethyl sulfoxide (DMSO).
3. Later, 1ml of the extract solution was pipette using a micropipette and dispensed into one of the three wells for each plate. 1ml of DMSO (negative control) was also pipetted and dispensed into one of the 2 remaining wells for both *S. aureus* and *E. coli*.
4. 1ml of tetracycline solution (positive control/standard for antimicrobial activity) was also pipetted and added to the third well on each culture plate.
5. Thereafter, culture plates were left 10 minutes for diffusion to take place and later incubated at 37°C for a period of 24 hours.
6. After an overnight, bacterial activity was seen by the formation of zone of inhibition which was measured using a ruler and recorded in millimeters (mm).

Determination of Minimum Inhibitory Concentration (MIC)

1. 5.5g of Mueller Hinton broth (MHB) was measured using an analytical weighing scale, followed by dissolving 100ml of distilled water, and sterilized using an autoclave at a temperature of 121°C for a period of 15 minutes, after which it was allowed to cool down.
2. Clean and sterile culture bottles were labelled clearly in two pairs, each pair with 6 tubes. One pair was labelled with *Staphylococcus aureus* and the other one with *Escherichia coli*. In each pair, the bottles were labelled with concentration in a 2-fold descending order from 1/2 up to 1/64 (Hartsock, Grewe *et al* 2022).
3. 1ml of the extract solution was pitted and transferred to a culture bottle 1 (1/2), mixed properly with a micropipette tip.
4. 1 ml of the mixture was withdrawn with a micropipette and transferred to bottle 2 (1/4).
5. Step 4 was repeated up to the 6th bottle where 1ml of the mixture was withdrawn from the bottle and discarded to maintain a uniform volume of the solution mixture. (Ansari *et al.* 2019).
6. 1ml of a solution of *Staphylococcus aureus* was pipetted and transferred to each of the 6 bottles in the first pair.
7. This procedure was repeated using a solution of *Escherichia coli* for the second pair.

Determination of Minimum Bactericidal Concentration (MBC)

1. Clean culture plates and Mueller Hinton agar solution where sterilized using an autoclave for a period of 15 minutes at a temperature of 121°C.
2. After cooling, the media was transferred to the culture plates.
3. Organisms that were from minimum inhibitory concentration (MIC), that is, from bottles with dilutions 1/2, 1/4 and 1/8 inoculated on the media for *E. coli*.
4. Step 3 was repeated for *S. aureus*.
5. The plates were then incubated for 24hrs to allow the growth of the test microorganisms.

Data analyses



GraphPad Prism version 8.0.0 was used to analyze experimental data (GraphPad Software, Boston, Massachusetts USA). Statistical means were determined and compared using one-way ANOVA and Turkey's multiple comparison test using mean zones of inhibition and standard deviation (Ezeabara *et al.* 2019).

RESULTS

Phytochemical screening results confirmed presence of 7 phytochemicals (alkaloid salts, tannins, phenols, coumarins, anthracenocides, saponins and steroid glycosides). Phenolic compounds showed an intense concentration, as indicated by the color intensity for their positive test results, whereas the rest 3 (anthocyanocides, reducing sugars and flavonoids) were confirmed absent [Table 1].

Table 1: phytochemicals that were confirmed present/absent in a methanolic extract of *Commelina diffusa* shoot.

Phytochemical	Observation	Deduction
Alkaloids	Pale brown	++
Saponins	Moderate foam	++
Tannins	Black solution	++
Phenols	Intense black solution	+++
Reducing sugars	No color change	-
Anthracenocides	Moderate color change	++
Anthocyanocides	No observable change	-
Coumarins	Pale yellow color	++
Flavonoids	No color change	-
Steroid glycosides	Pale green color	++

Commelina diffusa crude extract showed higher anti-bacterial effect on *E. coli* cells with an inhibition zone of 20.5mm and on *Staphylococcus aureus* cells with an inhibition zone of 18.5mm as compared to standard drug tetracycline (positive control) with inhibition zones of 16.0mm and 14.5mm in either case respectively. In both cases, there was no observable change for the treatment with dimethyl sulfoxide. [Table 2 & Figure 1].

Table 2. Antibacterial effect of the methanolic extract for *E. coli* and *S. aureus*.

Treatments	Zone of inhibition(mm)	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
DMSO	± 0.0	± 0.0



Tetracycline	16.0 ± 0.0	14.5 ± 0.0
Extract	20.5 ± 0.0	18.5 ± 0.0

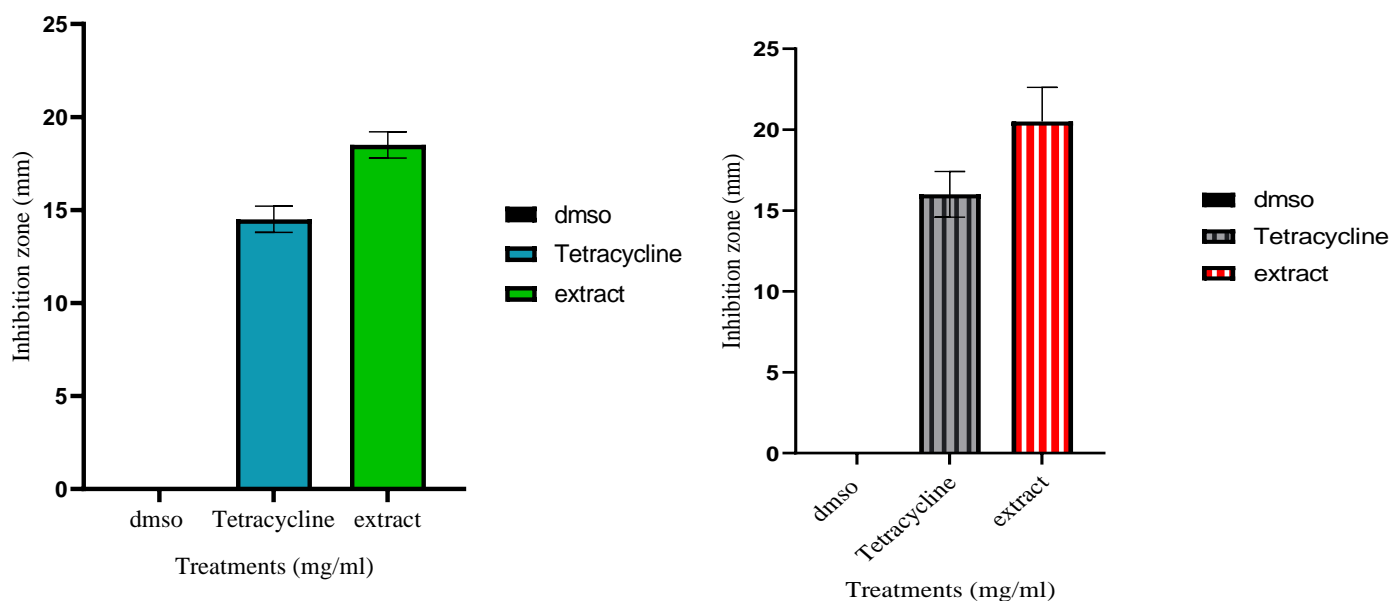


Figure 1. Relationship between zones of inhibition/activity and treatments for *Escherichia coli* (left) and for *Staphylococcus aureus* (right).

For both of two test microbes, minimum inhibitory concentration was 62.5mg/ml both while the minimum bactericidal concentration (MBC) was 62.5mg/ml and 125mg/ml for *E. coli* and *S. aureus* respectively [Table 3]. Antibacterial screening results carried out through ANOVA and Turkey's multiple comparisons tests are summarized in Tables 4 & 5 for *E. coli*, and Tables 6 & 7 for *S. aureus*. The results obtained were considered significant for *E. coli* and *S. aureus* at confidence levels of $p = 0.0016 < 0.05$ and $p = 0.0001 < 0.05$, respectively.

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract against test bacteria.

Organism tested	MIC (mg / ml)	MBC (mg / ml)
<i>Escherichia coli</i>	62.5	62.5
<i>Staphylococcus</i>	62.5	125

Table 4. ANOVA table for testing the significance of antibacterial activity of the plant extract against *E. coli*.

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
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Treatment (between columns)	464.3	2	232.2	F (2, 3) = P=0.0016 107.2
Residual (within columns)	6.500	3	2.167	
Total	470.8	5		

Table 5. Turkey's multiple comparisons test using mean zones of inhibition and standard deviation for *E. coli*.

	Mean Difference	95.00% CI of difference	Significant?	Adjusted P Value
DMSO versus. Tetracycline	-16.00	-22.15 to -9.849	Yes	0.0034
DMSO versus extract	-20.50	-26.65 to -14.35	Yes	0.0017
Tetracycline versus. extract	-4.500	-10.65 to 1.651	No	0.1079
Treatments			Inhibition zones (mm)	

Table 6. ANOVA table for testing the significance of antibacterial activity of the plant extract against *S. aureus*.

	SS	DF	MS	F (DFn, DFd)	P value
Treatment	379.0	2	189.5	F (2, 3) = 568.5	P=0.0001
Residual	1.000	3	0.3333		
Total	380.0	5			

Table 7. Turkey's multiple comparisons test using mean zones of inhibition and standard deviation for *S. aureus*.

	Mean Difference	95.00% CI of difference.	Significant ?	Adjusted P Value
DMSO versus. Tetracycline	-14.50	-16.91 to -12.09	Yes	0.0003
DMSO versus. extract	-18.50	-20.91 to -16.09	Yes	<0.0001
Tetracycline versus extract	-4.000	-6.413 to -1.587	Yes	0.0126



DISCUSSION

Regarding phytochemical composition, the result obtained is in line with a study conducted by Ekeke Chinezie, Chinedium. A and Alozie 2018, where total percentage concentration (g/100g) of flavonoids was higher in *C. erecta*(52.316%) than in *C. diffusa* (29.850%); and alkaloids higher in *C. diffusa* (8.765%) than in *C. erecta* (8%); phenolic acids higher in *C. diffusa* (52.344%) than in *C. erecta* and glycosides higher in *C. diffusa* (45.69%) than in *C. erecta* (38.71%) (Chimezie 2018). Phytochemical screening results for this study also agree with those obtained when a GC-MS methanolic study of phytochemical composition of *C. diffusa* plant shoot extract where the existence of 21 phytochemicals at varying percentage concentrations was confirmed present (Md Mizanur Rahman and Rajia Sultan, *et al* 2021) and that of an ethnopharmacological survey of medicinal plants traditionally used in Baham locality (Cameroon) using common phytochemical methods as described by Harbone (*C. et al.* 2017). In this study, several phytochemicals were found to be active on pathogenic bacteria suggesting that *C. diffusa* extract can be a promising antibacterial substance with a high potency. It has further been observed that *C. diffusa* shoot extract is a highly efficient antibacterial and this is in agreement with findings of many other recent studies conducted on the same plant like the one conducted by Ahad Ali Akan and his colleagues (Torequi and Samir Kumar 2011) who tested the methanolic extract against test microorganism *Escherichia coli* using standard disc diffusion method and obtained zones of inhibition ranging from 11mm – 19mm (Khan, Islam, and Sadhu 2011). This effect shows presence of antimicrobial compounds which can be extracted, separated and quantified for medical use against human pathogenic bacteria. Therefore, based on the results from the antimicrobial screening, the crude extract possesses significant antimicrobial activity that after rigorous higher scientific tests and pre- clinical investigations, can be confirmed to great importance to humans.

There is a significant relationship between percentage inhibition of *Escherichia coli* and *Staphylococcus aureus* and phytochemical composition of the *C. diffusa* extract ($p = 0.0016 < 0.05$) and ($p = 0.0001 < 0.05$) respectively (Tables 2 and 5). The antibacterial capacity of the plant extract is largely due to the presence of saponins, tannins, steroid glycosides, coumarins and phenols that are present in higher concentrations. The methanolic extract shows higher activity on *E. coli* as compared to *S. aureus*. This shows that the active components present in the methanolic extract of *C. diffusa* are more active on gram negative bacteria than gram positive bacteria. In this study, it was further observed that concentration of the active pharmaceutical ingredients in the extract is directly proportional to the degree of antimicrobial activity. At lower mass, there was less activity. However, when mass of the extract was doubled (from 125mg/ml to 250mg/ml), there was a significant increase in the activity of the methanolic extract on the test microorganism. It was also observed that the extract contained more oil and impure compounds as compared to pure ones which are responsible for low activity at low concentration.

Phytochemicals act by distortion proteins that make up the bacterial cell and eventually cause increase in cell permeability, thereby leading to leakage of cell contents, inhibition of expression



of cell surface proteins and interfering with certain microbial processes such as respiration that leads to loss of ATP energy, all of which cause cell death (Khameneh et al. 2021).

Furthermore, it was observed that the methanolic plant extract of *C. diffusa* showed moderate inhibition in both tubes containing *Escherichia coli* and *Staphylococcus aureus* 62.5mg/ml (table 7). In 2011 Md. Torequl Islam, Md. Ahad Ali Akan and Samir Kumar Sadhu conducted a study to evaluate the phyto-constituents and antimicrobial properties of *Commelina diffusa* and the results that were obtained are in line with the above results (Avp 2019). Standard drug tetracycline showed complete inhibition against the two test bacteria as compared to the turbidity/cloudiness in the negative control experiment where only media and bacteria were mixed and incubated. In the positive control, only media and extract were added but no bacteria and hence there was no growth and the mixture remained clear.

From this study, it was found out that the plant extract is bactericidal but not static, that is, Minimum Bacterial Concentration is 62.5 mg/ml and 125.0mg/ml for *Escherichia coli* and *Staphylococcus aureus* respectively. This shows lowest concentrations beyond which bacteria can grow. With *S. aureus* bacteria did not grow on all the three zones for the respective concentrations of 1/2, 1/4 and 1/8 and therefore MBC was taken to be 1/8 =125mg/ml bacterial colonies had grown on both zones, the extract would be bacteriostatic; however, it would inhibit growth.

CONCLUSION

This study showed a high significant level (p value < 0.05) between the Phyto-constituents and antibacterial potential of the methanolic extract which confirms a rich wealth of knowledge and usage of the plant by traditional health personnels and herbalists upon treatment of various bacterial infections. The in-vitro antibacterial potential of the plant extract supports its traditional use. This scientific evidence gathered shows that *Commelina diffusa* is a potential source of new antibacterial drugs. However, further investigations should be carried out to assess the quality and safety of the extracts in humans for future drug development.

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Haematological profiles and screening for selected haemoparasites among goats free of overt disease in Kassanda District, Uganda

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Abstract

Haematological evaluations are essential in the assessment of the physiological and health status of livestock like goats and cattle. Goats in this study, which appeared free of overt disease were found with circulating pathogens and deviating haematological profiles. The study aimed at detection of selected pathogens in goats free of overt disease. Blood samples were collected between June and November, 2023. Theileria, and babesia parasites, anaplasma and brucella species were screened for. Over 17.7% (n=28) of the goats presented with a disease causing organism while one doe had a mixed infection, although babesia parasites were not detected. Associations between the presence of the selected pathogens and haematological profiles were conducted using Fishers’ exact test. The study found a statistically significant association between reduced levels of lymphocytes and brucellosis (p = 0.018, 95% CI). A significant relationship was also found with increasing eosinophil levels in the presence of anaplasmosis (p= 0.024, 95% CI). There was no significant relationship between low MCH and brucellosis (p= 0.077, 95% CI). There was still no relationship between brucellosis and anaplasma infections with low HB (p= 0.079, 95% CI) and (p = 1, 95% CI), respectively. Similarly, there was no statistically significant relationship between brucellosis and anaplasma infections with PCV levels (p = 1, 95% CI), and



($p= 1$, (95% CI), respectively. MCV, MCH, and MCHC too had no significant differences in comparison with the reference. Being free of overt disease did not guarantee absence of circulating disease causing organisms and further still, being free of overt disease did not dictate normal ranges of haematological parameters. Furthermore, the presence of the pathogens detected did not statistically determine the haematological profile in the samples.

Key words: Goats, Haematological profiles, overt disease, Haemoparasites, Kassanda District

Introduction

Haematological evaluations are essential in the assessment of the physiological and health status of farm animals (1). Haematological profiles can also be conducted to establish the immunity status. However, the profiles could vary with pregnancy, nutrition and seasons (2). Goats are considered to be the second most important form of livestock in Uganda, with their very important roles in food security, and incomes to the communities, with additional cultural values. It is estimated that 39% of Uganda's households keep goats, an indication of the significance of goats to this population (3). The goats can easily be battered or turned into cash, and thus referred to as the poor man's cow (4). There are over 16 million goats in Uganda predominated by 90% of indigenous breeds like Mubende, Kigezi, and Small East African (SEA) goats (5). Goats are key in the production and productivity of food, maintenance of nutrition and economic security among developing countries(6). However, goat farmers in Uganda experience challenges such as ticks and tick-borne diseases, in addition to other diseases, nutrition, and dry season feed scarcity, informal markets and insufficient information flow on advanced management technologies (7).

Tick-borne diseases and brucellosis are among the major challenging diseases in this region. Brucellosis is a zoonotic disease which affects both humans and animals, causing immense loss to both animal production and human health (8). The burden of Brucellosis in Uganda, among small holder farmers is poorly documented, though anticipated to be high considering the high endemicity levels, enormous exposures and limited control measures (9). Haemoparasites are a key constraint in the livestock rearing since they impact the livestock production and productivity, since they are associated with disruption of the physiology of the animal (10), however, there is scanty information on haemoparasites among small ruminants in Uganda (11). There is a continuous exchange of goats through purchase from one farmer to another within and across Districts, and most of the indigenous goats among farmers are acquired through purchase, though some exotic goats are from Government interventions (5). The aim of the study was to screen for haemo-protozoan parasites, anaplasma and brucella among goats free of overt disease, and also establish their haematological pictures.

Anaplasmosis is a bacterial tick-born disease characterized by reduced livestock production with decreased milk yield, poor growth and enhanced susceptibility to other diseases (12). Anaplasmosis is also mechanically spread by contaminated blood, using needles, ear tagging or



dehorning equipment or biting flies(13). Anaplasma is an intra-erythrocyte infection that induces erythrophagocytosis and eventually leading to haemolytic anaemia. Animals which recover from the disease could remain life-time carriers, thus serving as reservoirs for other susceptible hosts (14)

Babesiosis is a tick-born protozoan disease registered for high morbidity and mortality in livestock in tropical and sub-tropical countries. It affects cattle, goats, sheep, hogs, cats, horses, and dogs. Babesiosis presents with fever, varying levels of haemolysis and anaemia. (15). It leads to reduced milk production, increased respiratory and heart rates, and lethargy. The disease can also cause abortion among pregnant cows and reduced fertility among bulls (16)

Theileriosis is a tick-born disease caused by haemoprotozoan parasites of the genus Theileria (17). Theileriosis affects both domesticated and wild animals. The disease causes significant morbidity and mortality among livestock and thus threatens production and productivity. Theileria are intracellular protozoan parasites which infect erythrocytes and lymphocytes, which may culminate into chronic anaemia. Kim (2017) (18) showed that theileriosis changed the erythrocyte and haematocrit profile among indigenous cattle in Korea. Several Theileria species are said to induce mild disease or remain asymptomatic (19).

Brucellosis is a zoonotic disease and a public health threat in several places around the world. It is caused by several species of the genus Brucella. Brucellosis is transmitted to animals through natural mating with infected males, artificial insemination with contaminated semen or through ingestion of infected aborted materials (20). Humans can contract brucellosis through consumption of contaminated raw animal products like meat, unpasteurized milk and milk products. Brucellosis leads to considerable economic losses due to abortion, infertility among livestock, reduced offspring production and low milk yield (21). The disease can lead to haematological abnormalities such as anaemia, thrombocytopenia, leucopenia, and pancytopenia, in the course of disease (22).

Materials and methods

Study area and design

A cross-sectional study was conducted in Kassanda District, which is located in the Central part of Uganda. The samples were collected from June to November, 2023. A total of 158 blood samples were collected from goats reared in different homesteads, communal grazing grounds and intensive production farms from goats which were free of overt disease. The samples were collected from the jugular vein into ethylenediamine tetra-acetic acid (EDTA) and another vacutainer with a clot activator. The samples were collected from five sub-counties Myanzi, Kitumbi, Nalutuntu, Kiganda and Manyogaseka (Figure 1). The samples were transported at 4°C to the laboratory at Makerere University, College of Natural Sciences- for further analysis.

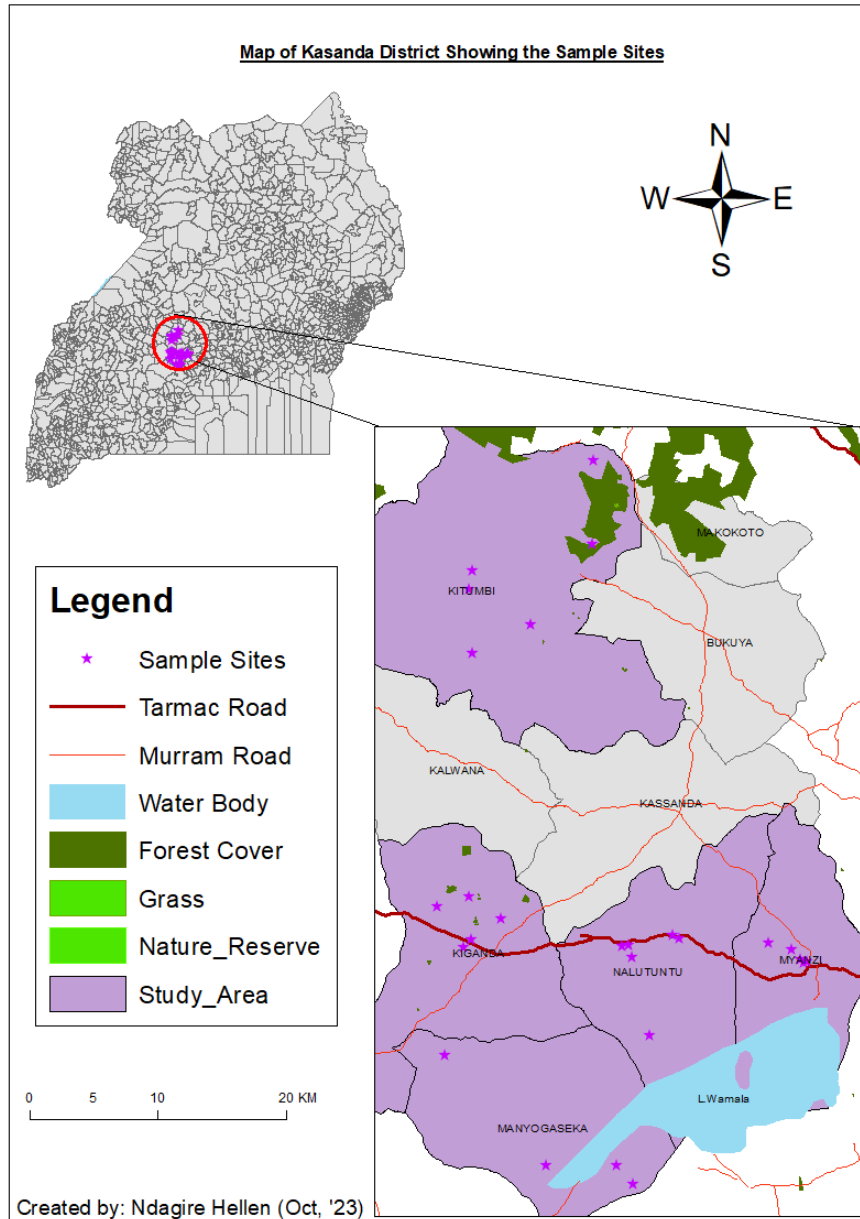


Figure 1: Map showing sampling sites in Kassanda District, Uganda

Haematology profiles

The samples were analysed for Packed cell Volume (PCV) using a Haematocrit (MRC-HCEN) within four hours of collection. Haemoglobin (HB) levels were estimated by colorimetry (MRC-HCEN-202). The samples were also analysed for other haematological parameters; total white blood cells, Lymphocytes, Neutrophils, and Eosinophils. Mean Corpuscular Volume (MCV),



Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) were also analysed by microscopy. Reference to the MSD manual of Veterinary Medicine was used to interpret the results observed.

Screening for haemoparasites

The samples were screened for three haemoparasites including Anaplasma, Babesia and Theirelia by microscopy. Further screening for Brucellosis was also done using the Rose bengal test.

Data analysis

Relationships between the haematological pictures and presence of an infection were analysed using Fishers' exact test. We took an alpha level of .05 for all statistical tests. Data was analysed using the R- programming language (Version 4.3.1).

Ethical considerations

The study received ethical approval from the Institutional Animal Care and Use Committee (IACUC) of the School of Veterinary Medicine and Animal Resources (SVAR), Makerere University; Reference number: SVAR-IACUC/97/2021, and a registration number A336ES, with the Uganda National Council for Science and Technology.

Results

Haemoparasites on microscopy

Microscopic examination showed 8.9% (n =14) anaplasmosis including 13 does and one buck. Theireliosis occurred in 1.9% (n= 3) of the goats tested, and 6.9% (n= 11) had brucellosis. Babesiosis was absent. Only one doe was found with mixed infections of anaplasmosis and brucellosis.

Haematological profiles

On testing all the collected samples, 14.6% (n=23) had low Packed Cell Volume (PCV) against the reference range. Low haemoglobin (HB) levels were recorded in 23% (n= 37) of the samples with only one goat presenting elevated HB. Elevated MCV was recorded in 0.63% (n=1) of the goats, while the rest were within the reference range. Elevated MCH was noted among 1.9% (n= 3) of the samples while 7.5% (n= 12) had low MCH, and 90.5% (n = 143) were within reference range. Elevated MCHC was observed in 3.8% (n=9) of the samples while 5.7% (n = 6) had low levels and 90.5% (n = 143) were within reference range. Lymphocytes were elevated in 3.8% (n= 6) of the goats while 24% (n= 38) had low levels and 72.2% (n= 114) were within reference range. The eosinophils were low in 17% (n=27) samples, while 7.6% (n=12) were elevated Neutrophils



were elevated in 8.86% (n= 14) of the goats, while 10.76% (n = 17) had low levels, 8.86% (n= 17) were elevated and 80% (n= 137) were in reference range. Infections were recorded in 17.7% (n=28) of the samples (Table 1).

Table 1: Table showing the Haematological parameter levels

Haematological parameter	Number of samples		
	Low levels	Elevated levels	Normal range
PCV	23	0	137
HB	23	1	120
MCV	0	1	137
MCH	12	0	157
MCHC	6	9	143
Total WBCs	6	16	83
Lymphocytes	38	6	114
Neutrophils	17	14	137
Eosinophils	27	12	119

Table 2: Haematological parameter levels in the presence of Anaplasmosis

Haematological parameter	Number of samples		
	Low levels	Elevated levels	Normal range
PCV	3	0	11
HB	3	0	11
MCV	0	0	14
MCH	1	1	12
MCHC	1	1	12
Total WBCs	1	2	11
Lymphocytes	6	0	8
Neutrophils	1	3	10
Eosinophils	0	3	11

Table 3: Haematological parameter levels in the presence of Brucellosis

Haematological parameter	Number of samples		
	Low levels	Elevated levels	Normal range
PCV	2	0	9



Haematological parameter	Number of samples		
	Low levels	Elevated levels	Normal range
HB	2	1	8
MCV	0	1	10
MCH	4	3	4
MCHC	1	2	4
Total WBCs	0	0	11
Lymphocytes	6	4	1
Neutrophils	2	0	9
Eosinophils	2	1	8

The statistical association between haematological profiles in the presence of disease

The Erythrocyte picture

There was no significant relationship between anaplasmosis and HB levels among the goats ($p=1$, CI = 95%, Odds Ratio, OR = 0). Similarly, no significant relationship existed between brucellosis and HB levels ($p = 0.079$, 95% CI, OR = 1) (Figure 4). No association was found between anaplasmosis and PCV ($p = 1$, 95% CI, OR = 0). Similarly, no association was drawn between brucellosis and PCV ($p = 1$, 95% CI, OR = 1). This study found no significant relationship between anaplasmosis and either MCV or Mean Corpuscular Haemoglobin (MCH) levels since all values were in the reference range. No significant association was found between brucellosis and MCV ($p=1$, 95% CI, OR = 0), or MCH (0.077, 95% CI, OR = 1), or MCHC ($p=1$, 95% CI, OR= 1.396), respectively. Similarly, no significant association was found between anaplasmosis and MCHC ($p=1$, 95%CI, OR = 0.65).

5The Leukocyte picture

A significant relationship was observed between elevated levels of eosinophils and anaplasmosis ($p= 0.024$, 95% CI) (Figure 2), yet there was no significant relationship between reduced eosinophils levels and brucellosis ($p= 1$, 95% CI, OR= 1.13). No significant relationship existed between anaplasmosis and elevated lymphocyte levels ($p=0.57$. 95% CI, OR =0). A significant relationship was observed between the reduced lymphocytes and brucellosis ($p= 0.018$, 95% CI, OR= 9.85) (Figure 3). However, there was no significant relationship observed between elevated lymphocytes levels and positive test of brucellosis. Also, no significant changes were observed in the levels of neutrophils in the presence of anaplasmosis, or brucellosis (Figure 5) and for theireliosis among all the samples.



Mosaic plot of Eosinophils in Anaplasmosis
Fisher's exact test, $p= 0.024$

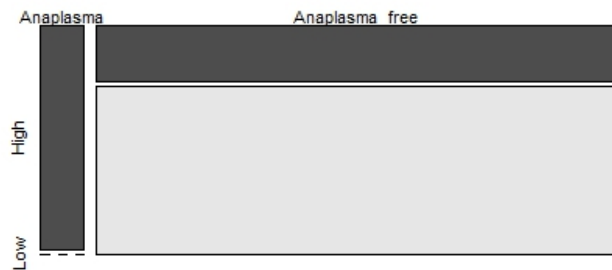


Figure 2: Representation of low Eosinophils in Anaplasmosis

Mosaic plot of Lymphocytes in Brucellosis
Fisher's exact test, $p= 0.018$

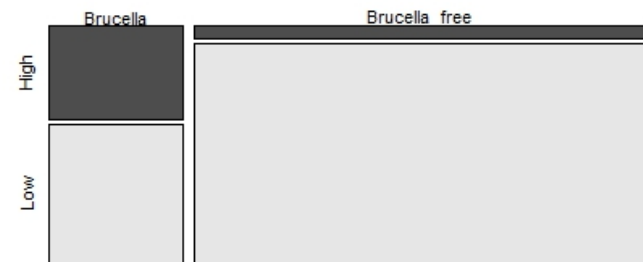


Figure 3: A representation of low Lymphocytes in Brucellosis



Mosaic plot of haemoglobin in Brucellosis
Fisher's exact test, $p= 0.079$

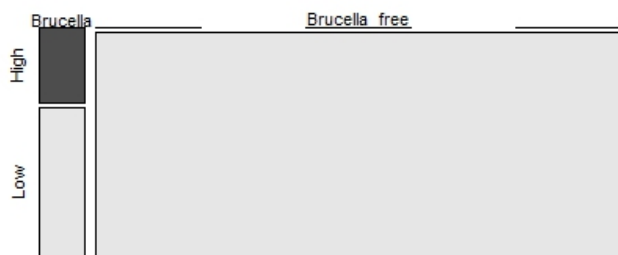


Figure 4: A representation of high Haemoglobin in Brucellosis

Mosaic plot of Neutrophils in Brucellosis
Fisher's exact test, $p= 0.488$



Figure 5: A representation of low Neutrophils in Brucellosis



Discussion

Presence of disease causing organisms and haematological profiles

Goats have been appreciated as hardy animals, acclimatised to inadequate temperature and humidity, in addition to poor pastures, which has given the goats an ability to adapt to hot and cold conditions in addition to disease resistance (23). A total of 158 goats that were free of overt disease were sampled, 17.7% (n=28) of them were found to be carrying either Theileria, Anaplasma, or Brucella causing organisms, despite the healthy presentation, an indication of disease tolerance (24). Among the sampled animals, several goats which were free of overt disease, and still tested negative for the screened diseases, still gave some parameters outside the reference range of the haematological picture. It is however, specified that there are several factors such as sex, age, breed, nutrition, weather, stress, and physiological state, which influence the blood parameters, since they modify the homeostasis of the animal (1).

The association between haematological profiles and disease presence

The study observed that 48% (n= 12) of the goats which tested positive for at least a disease causing organism had low lymphocytes as was displayed in the case of anaplasmosis (12). There was no statistically significant relationship between the low HB and PCV values and the presence of anaplasmosis and brucellosis among the sampled goats. This was in agreement with the findings in Bangladesh among cross- bred cattle, where no significant variations in blood parameters were observed between the positive and negative cattle for brucellosis (25). Similarly, with other haematological profiles namely the MCV, MCH, MCHC, total white blood cell count, and neutrophils, their diversion from the reference range had no statistical significance with the presence of brucellosis and anaplasmosis, justifying that the diversion was due to other factors (1). There was a significant increment in the levels of eosinophils in the presence of anaplasmosis similar to another study in Nigeria (26). Unlike the findings in this study which experiences changes with eosinophils in the presence of anaplasmosis. A study among ovines in Iran, did not find any change in the eosinophils. The same study in Iran still did not find any significant changes in the lymphocyte, and neutrophil levels among ovines in the presence of anaplasmosis (27). However, this study's findings showed a near to a significant relationship between low MCH levels ($p= 0.077$, 95% CI) and low HB levels ($p= 0.079$, 95% CI) in the presence of brucellosis. This was in agreement with a study in Colorado among experimental mice, where significant changes were observed with decreasing HB and MCH (30). Brucellosis has been reported to induce bone marrow suppression, thus leading to low red blood cell formation (28), hence the big ration of depressed levels of HB and MCH in the findings of the study. Similar findings were attained where consistent observations of decreased RBCs, HB and PCV were attained though with an increased MCV (29). The presence of diminished levels of lymphocytes in brucellosis displayed a statistically significant relationship, similar to the study in Colorado which displayed reducing lymphocyte in *B. arbutus* among experimental mice (30). The study among goats following a challenge with a virulent culture of brucella showed a variation from this study by observing increased lymphocytes (31). Increased lymphocyte levels are suggestive of cellular response to the infection (32). However, the study in Egypt among cows and ewes acquired no significant changes in lymphocytes among cattle and ewes during brucellosis infection (33). Leucopenia was also observed among brucellosis patients (34).



Conclusion

Among the sampled goats, 17.7% of the goats tested positive for haemoparasites, much as they were free of overt disease. The haematological profiles of the sampled goats did not have significant statistical associations with the haemoparasites screened except for the low levels of lymphocytes in brucellosis and elevated levels of eosinophils in the presence of anaplasma causing organisms. It alludes that the contribution of other factors, including but not limited to other infections not screened for in this study could have played a role in the haematological profiles of the sampled goats.

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Smallholder farmers' knowledge, attitude and practices towards use and disposal of veterinary antibiotics in Kassanda District, Uganda - A cross sectional survey

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Abstract

Global health is threatened with a potential post-antibiotic era due to antimicrobial resistance with a raging pandemic by 2050. Misuse and over use of antimicrobial agents are the major selective pressures for antimicrobial resistance (AMR) in humans and animals. Inadequate knowledge, attitude and practices are the feed mills of misuse and overuse of antimicrobial agents. The study aimed at assessing smallholder farmers' knowledge, attitude and practices towards antibiotic use and disposal in Kassanda District. A self-administered questionnaire was distributed among 378 respondents in Kassanda, a rural administrative District in Uganda. The study enrolled 378 respondents (female, n=205; male, n=173). Almost half of the population was between 25 and 45 years of age, 24% were between 45 and 65years, 16% were over 65 years old while 11% were between 18 and 24 years. The levels of education were tertiary, advanced, ordinary, and primary distributed as 6%, 3%, 25%, 53%, and 13% illiterate respondents, respectively. Poor practice towards antibiotics use was noted among 21% of respondents yet 62% had administered antibiotics to their animals in the previous twelve months. The use of critically important antibiotics was noted in two chicken rearing households. Self-treatment was reported by 49% respondents, varying with level of education ($p = .017$). Many respondents sold the animals upon failure of antibiotics (46%) while others consumed the meat (32%) among other behaviours. Inadequate awareness was noted about antibiotic withdraw messages on packaging materials (69%) varying with education level attained ($p = 4.079e-06$), withdraw periods upon administration of antibiotics (49%), poor drug disposal methods (77%) and poor antibiotic-use record keeping (95%) as contributors to the spread of AMR. Poor attitude towards consumption of animal products whilst the animals were on antibiotics, safe disposal of used and expired antibiotics, establishment of drug return programmes, administration of human antibiotics to animals, self-prescribing behavior, and legal restriction of the purchase of antibiotics was exhibited (73%). Some demographic characteristics were significantly associated with knowledge, attitude and practices.

Keywords: knowledge, attitude, practices, veterinary antibiotics, resistance, disposal, Kassanda

Introduction

Antimicrobial resistance threatens the world with a potential post-antibiotic era shrouding the globe with a possible pandemic by 2050(1). Antimicrobial resistance (AMR) is associated with 4.9m deaths annually (2). Similar to global reports, the incidence of AMR is on the rise in



Uganda and Africa at large. Misuse and over use of antimicrobial agents is the primary selection pressure driving the global spread of antimicrobial resistance (AMR) in humans and livestock(3). Lack of access to safe water, proper sanitation and basic hygiene for both humans and animals equally contribute to spread of antimicrobial resistance (2). However, physical and economic water scarcity associated with rapid population growth and climate change still exists in Africa and many other low and middle income countries. Large proportions of the human and livestock populations consume unsafe water (4). Lack of access to quality, affordable antimicrobials, vaccines(5) and diagnostic tools lead to spread of AMR, too (6). Also, the advent of AMR is associated with lack of awareness and knowledge, poor attitudes and practices among antimicrobial users and the lack of legislations and their enforcement (6) particularly in developing countries. The lack of provisions and policies for proper drug disposal contaminates the environment with suboptimal doses with antimicrobial agents thus facilitating the emergence of AMR.

Antimicrobial misuse and overuse lead to emergence of resistant microorganisms including bacteria, fungi and protozoa that can be transferred to humans through the food delivery systems or direct contact (7). This study aimed at assessing smallholder farmers' knowledge, attitudes and practices towards antibiotic use and disposal in Kassanda District, Uganda.

Materials and methods

Study design, area and period

A cross sectional study was conducted through a self-administered questionnaire to assess knowledge, attitudes and practices of smallholder farmers as antibiotic users and handlers in Kassanda District, Central Uganda (Figure 1). The study was conducted between June, 2022 and September, 2023.

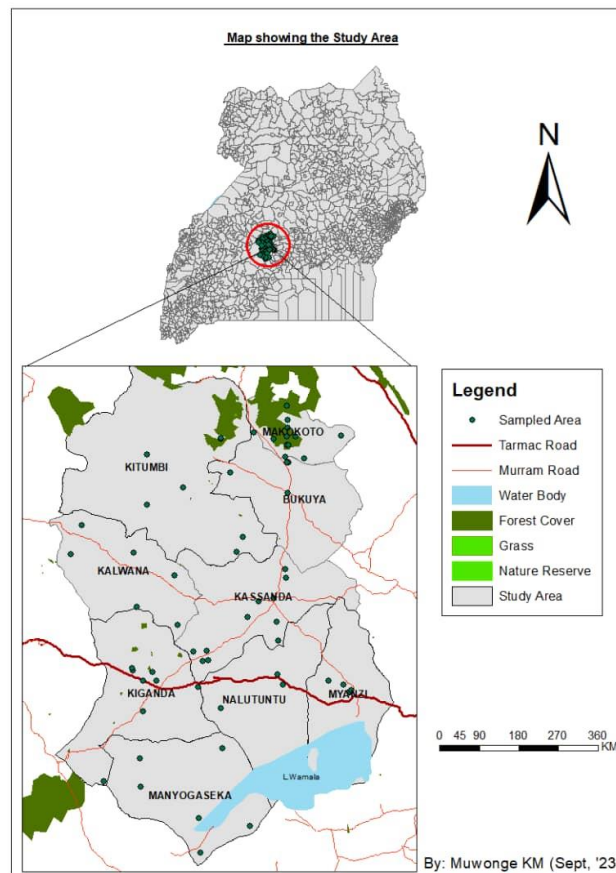


Figure 1 Map showing the location of the study area

Sampling design and strategies

Stratified sampling was employed to select participating households in Kassanda District. The first household in each parish was randomly selected and every 5th household was selected until the sample size was attained. A standardized questionnaire prepared in English was translated to Luganda, the local language. The questionnaire was pre-tested on 15 households and these data were not considered in the study. The questionnaire consisted of socio- demographic characteristics, awareness, attitude, and practices of farmers in handling veterinary antibiotics.

Inclusion criteria

Only livestock keeping household members above the age of 18 years, without mental illnesses were included in the study. Household members with speech or hearing impairment were excluded from the study.

Ethical approval

Ethics approval for this study was received from School of Veterinary and Animal Resources Institutional Animal Care and Use Committee (SVAR_IACUC/83/2021) and registered with UNCST/A187ES.

Demographic variables



The demographic variables included gender, age, and highest level of education. The KAP section for farmers included four questions about knowledge of antibiotic use and resistance and efficient drug disposal (Table 1). Attitudes towards the drivers of antibiotic resistance and safety of drug disposal methods were measured by six questions asking respondents about consumption of animal products during treatment, use of human antibiotics in animals, self-prescription, legal restriction of purchase of antibiotics, their confidence in the methods used to dispose drugs at household level and their willingness to participate in drug return programmes. (Table 2). The practices and behaviours of respondents were assessed through 23 questions.

Data analysis

R programming language (version 4.3.1) was used to produce preliminary contingency tables of the variables representing knowledge, attitudes, practices and demographic characteristics of the respondents. We used Pearson's Chi-squared test to establish bivariate relationships of the different variables. We used an alpha level of .05 for all statistical tests.

Results

Social-demographics

The study enrolled 378 participants with 100% response rate (female, n=205; male, n=173), of whom 42 were aged between 18 and 24 years, 185 were between 25 and 44 years, 91 were between 45 and 64 while 59 were over 65 years old. The levels of education were tertiary, advanced, ordinary and primary with 24(6%), 10(3%), 94(25%), and 202 (53%) respondents, respectively. The illiterate respondents were 48(13%).

Assessment of practices towards antibiotics use and handling

Our data show that most respondents adhered to good practices while using antibiotics (78.8%, 298/378). However, 21.2% expressed poor practices towards antibiotics use and handling in livestock health management. The major reason for administering antibiotics in animals was treatment for disease (95%, 258/272) while prevention of disease was noted in 5% (14/272) of respondents. Majority of the respondents (74.3%, 281/378) agreed to having ever administered an antibiotic to their animals, and 61.9% (232/374) had administered antibiotics to their animals in the previous twelve months. The deliberate use of antibiotics as growth promoters was not reported by any respondent. However, the administration of colistin in combination therapies added to drinking water was noted in two (2/378) chicken rearing households. Most respondents agreed to having sought veterinary advice (73.4%, 273/372) prior to purchase of antibiotics as compared to 26.6% (99/372) who did not. When the respondents were asked if they sought advice elsewhere other than that of a veterinarian/para-veterinarian before purchasing antibiotics, one hundred thirty three (36%, 133/372) agreed to doing so. We noted effectiveness as the major factor considered by the respondents (68%, 219/320) when purchasing antibiotics. One hundred two (32%) considered price as the major factor. The other factors considered by the respondents when purchasing antibiotics were recommendation of a veterinarian (77%, 245/318), distance to travel to an agrovet shop (13%, 41/318), quantity per unit cost (6%, 20/318), and length of expiry date (4%, 12/318), Figure 2. These frequencies were significantly different with age, $X^2(12, N = 318) = 12.497, p = .406$. Veterinary advice was mostly sought by respondents below 45 years of age, however, this age group considered distance travelled to an agrovet shop as a key determinant of their decision making to purchase antibiotics. When the respondents were asked for reasons for choosing where to buy antibiotics,



they referred to convenience of the place (54%, 172/320), veterinary advice (36%, 115/320), cost of the drug (6%, 19/320), and availability of a variety of drugs (0.9%, 3/320) among other reasons. When asked about their veterinary-advice seeking behaviour, 273(73.4%) responded in affirmation while 99 (26.6%) sought it from elsewhere including relatives, experienced farmers or their neighbourhood. When asked the reason for choosing where to buy antibiotics, majority indicated convenience of the place (n=172, 54%) including accessibility. Availability of drugs or veterinarian assistance was preferred by 115(36%) respondents. Others chose the place based on availability of 'special drugs' or more effective drugs (3.5%), and cost of the drugs (6%) or proximity to human drug shops (0.3%). Most respondents (n=251, 66%) did not have an agrovet shop within 3km from their place of residence. One hundred twenty seven respondents (34%) reported the presence of Agrovet shops within 3km from their households. Self-treatment was reported by 164(49%) respondents while 174 (51%) did participate in self-prescription for their animals. We noted variation in self-prescribing behavior with level of education (tertiary 13/20, advanced 2/8, ordinary 49/81, primary 87/183, and illiterate 13/41), $X^2(4,335) = 12.085$, $p = .017$. Chi-squared test did not establish a link between gender and self-prescribing behavior, $X^2(1,335) = .013$, $p = .909$. Using Pearson's Chi-square test, we did not find any association between self-prescribing behaviour and the distance travelled to an Agrovet shop, $X^2(2, N = 335) = 2.3$, $p = .315$. Only 19 (5%) respondents kept records of the antibiotics used, while 358 (95%) did not.

When asked if the respondents stop giving an antibiotic and save it for later use when the animal appears to get better before the end of full treatment, one hundred thirty six (42%) agreed to this practice while one hundred ninety six (58%) did not. When asked for their actions upon purchase of one type of antibiotics to treat an animal with and it does not work, we noted that majority sold the animal (45.6%, 52/114). Other respondents resorted to consuming the animal (32%, 36/114), changed the course of treatment (7%, 8/114), sought veterinary advice (6%, 7/114) or repeated the treatment (0.2%, 1/114) among other options (8.8%, 10/114). We found that majority of the respondents sold the animal products (meat, eggs, milk and milk products) obtained from an animal before the withdraw period of antibiotics had elapsed (50%, 169/336). We noted that other respondents discarded the meat, eggs, milk or milk products (22%, 73/336), or fed the milk to dogs or cats or calves in their households (4%, 13/336). The rest of the respondents had not experienced this (24%, 81/336). One hundred thirty eight respondents (38%) had ever purchased antibiotics intended for human consumption and administered it to animals (n=138/368). We observed that majority of the respondents had not vaccinated their animals (66%, 247/373) while others had their animals vaccinated (34%, 126/373).

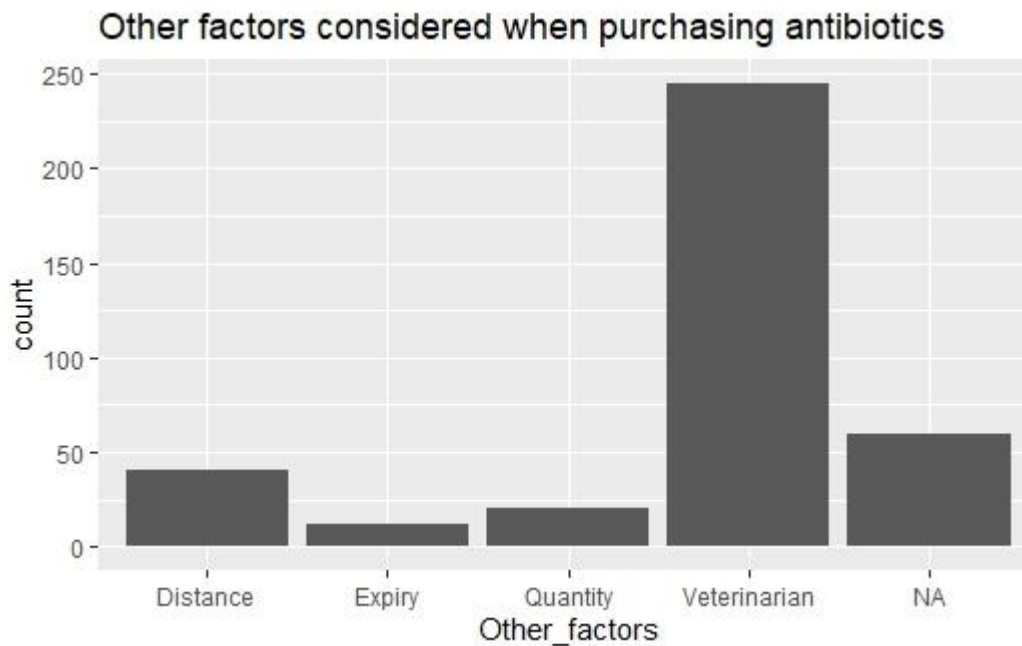


Figure 2 Other factors considered by respondents prior to purchase of antibiotics

*Count = Frequency, NA = Not applicable

Majority of the respondents kept leftover antibiotics (125, 56%) while others discarded the drugs (100, 44%). However, when respondents were asked if they save the antibiotics for later use when the animal gets better before completing the dose, 136 (42%) affirmed.

Veterinarian administration of antibiotics rated at 44% (143), estimation and following of veterinarian/agrovet shop attendants or para-veterinarian's advice rated at 134 (42%) while weight estimation and following instructions on packages was done by 14 (4%) respondents. Only one respondent (0.3%) performed weight estimation to determine the dosage by using a weighing band. Thirty one (9.7%) respondents did not follow any instructions prior to administering the antibiotics. The other ways of estimating animal weights prior to drug administration were based on age of the animal (114, 35%), veterinarian estimating the weight of the animal by simply looking at it (80, 25%) while those who lacked alternative methods were 132 (40%).

Antibiotic resistance was claimed to have occurred by 35(10%) respondents. The most reported conditions (symptoms or signs) presumed to be expressing antibiotic resistance were 'cough' (14, 44%), 'diarrhoea' (4, 12%), nasal discharge (4, 12%), 'abscesses' (3, 9%), 'dysentery' (2, 6%), 'post-abortion infections' (2, 6%), 'Fever' (2, 6%), and 'New Castle disease' (1, 3%) (Figure 3). Most resistance was claimed in poultry (20, 62%). Also, resistance in cattle (5, 16%), goats (4, 12%) and swine (3, 9%) was reported. When the respondents purchased a particular type of antibiotic and it was not effective, 53 (46%) sold the animal products, 36 (32%) slaughtered and consumed the meat while 8(7%) changed the course of treatment, 7 (6%) invited the veterinarian for reassessment, only one repeated the treatment while others left the animals without further treatment. We established a link between gender and respondent's behaviour upon failure of antibiotics, $X^2(5, 114) = 10.759, p = .05$. More female



respondents assented to consumption or sell of animal products upon antibiotic resistance. We found that increasing levels of education attained resulted in decreasing consumption or sell of animal products while the animals were still on antibiotics, $X^2(20, 114) = 41.629, p = .003$, with adjustment for multiple testing with Bonferroni correction.

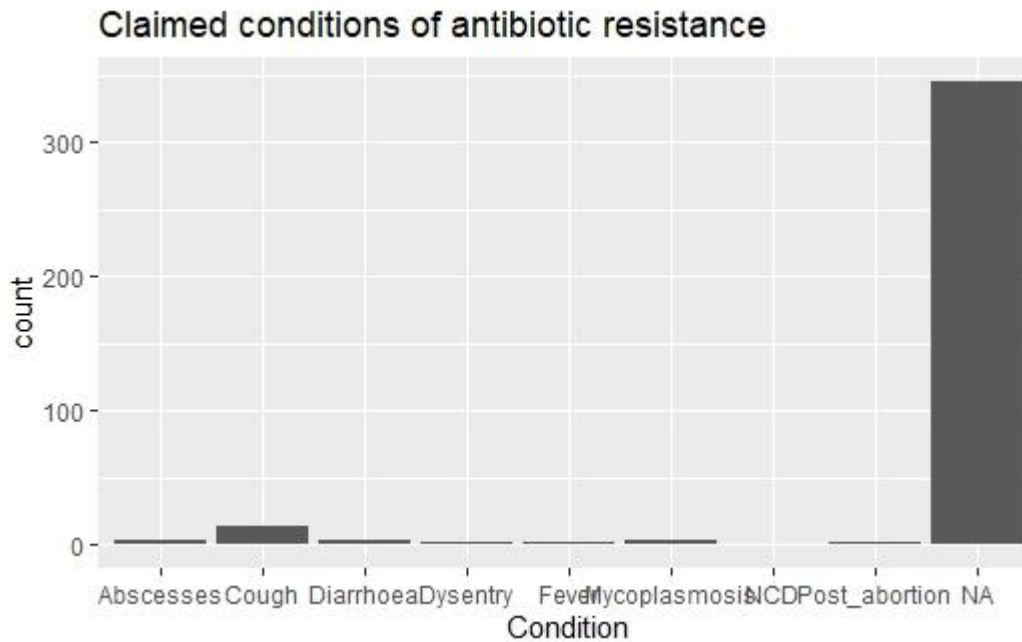


Figure 3: Claimed conditions of antibiotic resistance by respondents

*Count = Frequency, NA = Not applicable

Forty eight percent (170/352) of the respondents agreed to sell of meat, milk, milk products or eggs while the animals were still on antibiotics or prior to withdraw period. When asked what the respondents do with the animal products while the animals are still on antibiotics or prior to end of withdraw period, 50% (169/338) agreed to selling the products, 1% (4/338) fed the milk to calves, 21.5% (73/338) discarded the milk or eggs, 2.5% (9/338) fed the milk to domesticated dogs and cats, while it had not yet happened to 25% (83/338) of the respondents.

When asked if they had ever purchased antibiotics intended for human consumption and administered them to animals, 38% (138/368) agreed while 62% (230/368) had not. Ampicillin was the most misused human antibiotic in livestock (23%, 30/129), followed by Septrin (Cotrimoxazole, 20%, 26/129), Chloramphenicol (19%, 25/129) and Ampiclox (18%, 23/129). The other human antibiotics administered by the respondents were amoxicillin, ciprofloxacin, tetracycline, streptomycin, gentamycin and erythromycin at 9%, 5%, 3%, and 1%, respectively (Figure 4).



Pie Chart of Human drugs used

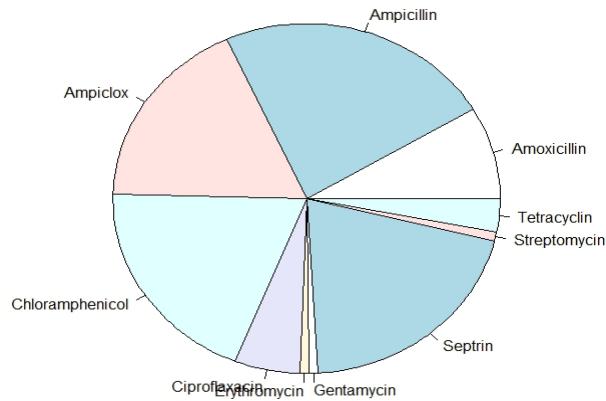


Figure 4: Pie chart of common human drugs used in the livestock industry

The reasons put forward for using human drugs instead of veterinary antibiotics were availability of the drug from drug shops or leftover human medicines (56%, 60/107), effectiveness of the antibiotics (25%, 27/107), the antibiotics are inexpensive as compared to veterinary services (13%, 14/107), and others used them because they are similar drugs thought to be of same effectiveness (6%, 6/107).

Discarding of unused and expired antibiotics into backyard gardens or bushes was the leading method of drug disposal (61%, 180/297). This was followed by open-air burning of medicines together with domestic waste (20%, 59/297), discarding into pit latrines (14%, 41/297), and disposal into garbage bins (mostly in townships (6%, 17/297). Incineration was never practiced by all respondents (Figure 5).

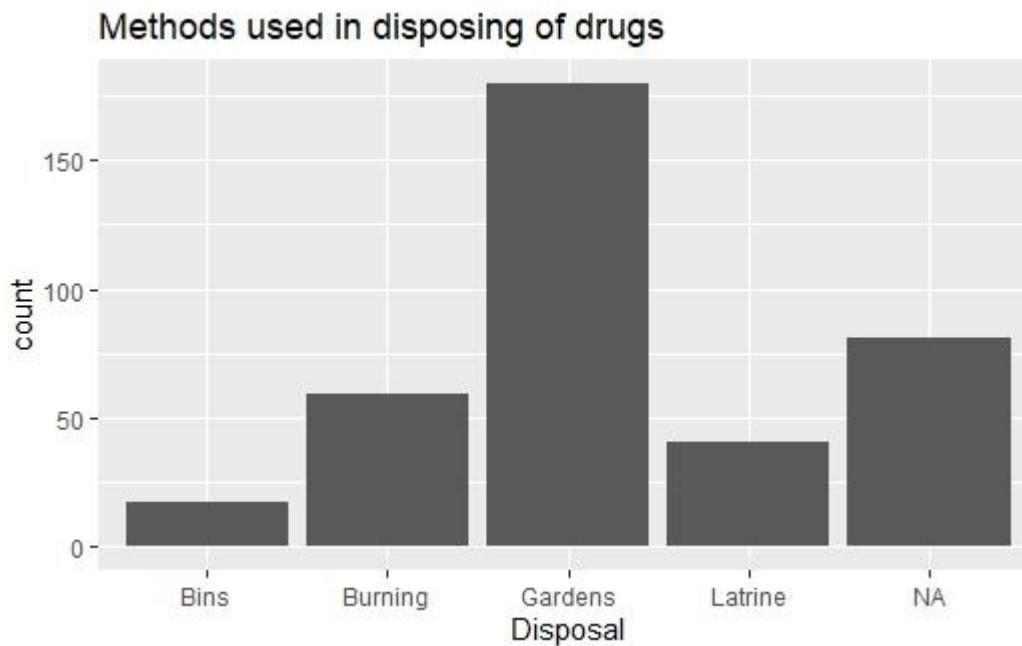


Figure 5: Methods used in disposing of drugs in Kassanda

*Count = Frequency, NA = Not applicable

We found an association between education level attained and the method of disposing unused and expired antibiotics, $X^2(12, 297) = 36.563$, $p = .0002$, where Bonferroni adjustment indicated that those with tertiary education mostly burn while those with ordinary level mostly use the latrine.

Most respondents had unvaccinated animals (66%, 247/374) while 34% (126/374) had at least a vaccinated animal in their household. Of those who had vaccinated animals ($n=130$), poultry (mostly chicken) were the most vaccinated livestock against Newcastle disease (58), Gumboro and other diseases included in the poultry schedule such as fowl typhoid, fowl pox, Marek's disease (fowl paralysis), and infectious bronchitis (19). Other vaccinations reported were foot and mouth disease (FMD) in cattle (27), Brucella in cattle and goats (04), and East Coast Fever (ECF) in cattle (01). Fifteen respondents had their veterinary service providers vaccinate the animals without participants keeping the records.

The major source of water for animal use and hygiene was unprotected wells or springs (42%, 155/370), followed by protected wells (31%, 116/370), other water sources including drilled wells or dug outs (9%, 36/370), flowing water (tap water, 8%, 28/370), rain water (4%, 16/370), swamp (4%, 13/370) and riverside water (2%, 6/370).

Assessment of knowledge of respondents towards use of antibiotics and disposal

Our data show inadequate awareness of respondents about antibiotic withdrawal messages on packaging materials (69%), withdrawal periods upon administration of antibiotics (49%), poor drug disposal methods (77.2%) and poor antibiotic-use record keeping (95%) as contributors to antibiotic resistance. Each response was scored as 1 (yes) or 0 (No or I do not know) to give a scoring range of 0% (minimum) to 100% (maximum). A score ≤ 55 was regarded as



inadequate awareness while that >55 was adequate. We noted that 83.3% (315/378) of respondents had inadequate knowledge of the evaluated variables against 16.7% (63/378). Sixty nine percent of respondents (259/378) were unaware of withdraw messages on packaging materials of antibiotics. When we conducted Pearson’s Chi-squared test with Yate’s continuity correction, no association was found between gender and awareness of withdraw messages on packaging materials, $X^2 (1, 378) = 0.205, p = .650$. We suggested an association between education level attained and awareness of withdraw messages on packaging materials, $X^2 (4, 378) = 30.388, p = 4.079e-06$. We noted that respondents with primary level of education were more likely to be unaware of antibiotic withdraw information on packaging materials. Fifty one percent (194/378) of the respondents had adequate knowledge about withdraw period of antibiotics while 49% (184/378) were unaware of it. Using Chi-squared analysis, we found no link between age of respondents and awareness of withdraw period of antibiotics, $X^2 (4, 377) = 3.125, p = .537$. Also, there was no correlation between gender and awareness of antibiotic withdraw period among respondents, $X^2 (1, 378) = 0.125, p = .723$ using Pearson’s Chi-squared test with Yate’s continuity correction. However, we noted an association between education level attained and awareness of withdraw period of antibiotics, $X^2 (4, 378) = 16.44, p = .002$, with Bonferroni adjustment. When asked if they are aware that poor drug disposal methods could contribute to antibiotic resistance, 77% (292/378) were unaware while 23% (86/378) were knowledgeable (Table 1).

Table 1 The Respondent’s Awareness of Antibiotics’ handling and Disposal in Kassanda District, Uganda, N = 378

Attribute	Response	
	Yes	No
	N (%)	N (%)
Awareness of farmers about antibiotic withdraw messages on packaging material	119(31.5)	259(68.5)
Awareness of farmers about withdraw periods upon administration of antibiotics	194(51.0)	184(49.0)
Awareness about poor drug disposal methods as contributors to antibiotic resistance	86(22.8)	292(77.2)
Awareness about poor antibiotic-use record keeping as a lead to antibiotic resistance	19(5.0)	359(95.0)

Assessment of attitudes of respondents towards use of antibiotics, handling and disposal

Our data show most respondents had poor attitude (72.5%, 274/378) towards consumption of animal products whilst the animal is on antibiotics treatment, safe disposal of used and expired antibiotics, establishment of drug return programmes, administration of human antibiotics to animals, self-prescribing behavior, and legal restriction of the purchase of antibiotics. Each response was scored as 1 (yes) or 0 (No or I do not know) to give a scoring range of 0% (minimum) to 100% (maximum). A score ≤ 55 was regarded as negative attitude while that >55 was positive. Most respondents (74%, 257/346) believed that their method of drug disposal was safe, 12% (43/346) new it was unsafe, 13% (46/346) did not know whether the method is safe or not. When asked if the respondents would return unused and expired antibiotics to drug



shops or pharmacies or Agrovet shops or clinics for proper management and disposal, 45% (165/366) responded to the contrary (Table 2).

Table 2 The Respondent's Attitude Towards Use of Antibiotics, handling and Disposal in Kassanda District, Uganda, N = 378

Antibiotic Use, Resistance and Disposal items	N	%
Do you think antibiotic resistance can increase through consumption of animal products (meat,/cheese/milk/eggs) whilst the animals are still on antibiotics or have recently finished treatment		
Yes	185	48.9
No/I do not know	193	51.1
Do you think it is safe to dispose medicines the way you do?		
Yes	91	24.0
No/I do not know	286	76.0
If you were asked to take unused and expired antibiotics to pharmacies/agrovet shops or clinics, would you take them?		
Yes	201	53.0
No	177	47.0
Do you think giving human antibiotics to animals can increase the risk of antibiotic resistance?		
Yes	267	70.8
No/I do not know	110	29.2
Do you think self-prescribing of antibiotics in animals can increase the risk of antibiotic resistance?		
Yes	121	32.1
No/I do not know	256	67.9
Should the purchase of antibiotics from drugshops for animal use be restricted legally?		
Yes	156	41.4
No	221	58.6

Discussion

In the present study, 62% of respondents had administered antibiotics to animals under their care in the previous twelve months. These findings are lower than 79 and 72.8 percent use of antibiotics reported in Ejere District of Ethiopia(8) and in Kuwait(9), respectively. In the current study, most farmers (78.8%) adhered to good practices while using and handling antibiotics. However, 21.2% expressed poor practices towards antibiotics use and handling in livestock health management. These findings are lower than 60.88% reported in Saudi Arabia(10). The poor practices included failure to adhere to antibiotics withdraw periods and thus selling or consuming the meat, eggs, milk or milk products, discarding milk in the environment or giving it to dogs and cats while the farm animals were still on treatment. Similar poor practices were reported in India(11). Other poor practices were self-prescribing behavior, using antibiotics in prophylaxis or growth promotion, searching for veterinary advice from



peers, purchasing antibiotics based on price, distance to travel, or quantity per unit cost other than effectiveness, recommendation of a veterinarian or length of expiry date. Also, administering human drugs to livestock, open-air burning of drugs with domestic garbage, discarding drugs in nearby/backyard gardens or bushes or latrines. We also noted saving the antibiotics for later use if the animal appears to get better before the end of full treatment, and poor methods of working out the correct dosage. Unfortunately, the levels of vaccination in livestock were as low as 34% though higher than the findings in Ejere District, Ethiopia at 21%(8). Vaccination is crucial in the fight against antimicrobial resistance in both animals and human beings as it reduces the volume of antibiotics used (5).

This study found penicillins as the most misused human antibiotics in livestock at 50% followed by sulphamethoxazole-trimethoprim (20%), chloramphenicol (19%), ciprofloxacin (5%), tetracycline (3%), streptomycin, gentamycin and erythromycin were each at 1% as the least used. Similar reports of administering human antibiotics in livestock health management were noted in Ethiopia(12). In our study, the reasons for use of human antibiotics were accessibility of drug shops as compared to agrovet shops in remote areas. Other respondents stated that human antibiotics are inexpensive as compared to veterinary services. Also, resorting to leftover human antibiotics after treating family members was recorded. The use of human antibiotics in livestock drives antimicrobial resistance that affects public health (12).

Sadly, most respondents (69%) were unaware of withdraw messages on packaging materials of antibiotics. Our findings indicate a higher percentage than that in Zaria, Nigeria where half of the participants had never heard of withdrawal period of drugs(13). In our study, the deliberate use of antibiotics as growth promoters was not reported by any respondent. However, the administration of colistin, a polymixin and growth promoter in chicken was reported in combination therapies added to drinking water in two chicken rearing households. Colistin is a critically important, dernier-resort molecule in the treatment of multidrug-resistant Gram-negative bacterial infections designated by WHO(14). The use of critically important antibiotics in regular drinking water episodes with subsequent likelihood of developing resistant strains is a threat to both human and animal life. Use of growth promoters in livestock has also been reported in the United Kingdom much as it had been prohibited(15). Therefore, enforcement is required to address the use of critically important antibiotics in growth promotion or disease prevention in livestock in Uganda.

In the present study, veterinary or para-veterinary advice was not sought by 36% of respondents prior to purchase of antibiotics, also 36%, agreed to consulting peers. Similar findings were reported in India(16). Treatment with antibiotics was based on previous experience by the individual or peers who had earlier on used the same treatments for similar signs and symptoms, just like self-treatment has been reported among human beings in Cameroon, thus heightening antibiotic resistance(6,17)

This study showed that 32% of the respondents considered price against effectiveness as the major factor when purchasing antibiotics. In a similar way, consideration of quantity per unit cost prior to purchase of antibiotics was recorded at 6%. It is saddening that farmers consider price or quantity per unit cost other than effectiveness since these could enhance under dosing and general misuse of antibiotics.



In this study, only 5% of respondents kept records of the antibiotics used to treat the animals thus a challenge to veterinarians who treat the respective animal on subsequent events. Also, poor record keeping of antibiotics used makes it difficult for AMR surveillance (18).

Our respondents reported presumed antibiotic resistance in form of ‘cough’ (44%), ‘diarrhoea’ (12%), nasal discharge (12%), ‘abscesses’ (9%), ‘dysentery’ (6%), ‘post-abortion infections’ (6%), ‘fever’ (6%), and ‘New Castle disease’ (3%) that are non-definitive infections. Similar illnesses were reported in India (19). Antibiotic misuse is occurring in treating viral infections or signs and symptoms of bacterial or fungal infections without proper diagnosis. Self-treatment is facilitated by over-the-counter antibiotics available in either agrovet shops or human drug shops in the country.

The present study found respondents who sold meat, milk, milk products or eggs while the animals were still on antibiotics prior to withdraw period (48%). Others fed the milk to calves, dogs or cats in the household, or discarded milk in the environment. Antibiotic residues exist in animal products that are consumed or discarded in the environment prior to withdraw period of antibiotics (20) yielding sub-optimal levels of antibiotics that breed antimicrobial resistance (6). Lack of access to safe water for animal consumption and adequate sewage disposal can spread AMR. From our respondents, we established that the major source of water for animal use and sewage disposal was unprotected wells or springs (42%), protected wells (31%), other water sources included drilled wells or dugouts (9%), tap water (8%), rain water (4%), swamp or riverside water (6%). Appropriate policies should be drafted to monitor the safety of water sources that could spread resistant strains or resistance genes through the livestock value chain in Uganda.

We found an association between education level attained and awareness of withdraw messages on packaging materials ($p = 4.079e-06$). We noted that respondents with primary level of education were more likely to be unaware of antibiotic withdraw information on packaging materials. Also, we noted an association between education level attained and awareness of withdraw period of antibiotics ($p = .002$). This explains the relevance of universal education in Uganda to create awareness in the fight against disease including AMR. This study found inadequate attitudes, awareness and practices among respondents towards antibiotics use, handling and disposal. Enforcement is needed to address the use of critically important antibiotics in growth promotion or disease prevention and treatment in livestock in Uganda. The Government of Uganda may establish appropriate awareness programmes to combat the poor attitudes and practices, and thus create awareness of antibiotic resistance.

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LOCAL PEOPLE’S KNOWLEDGE, ATTITUDES, AND PERCEPTIONS OF OIL AND GAS EXPLORATION IN MURCHISON FALLS NATIONAL PARK

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ABSTRACT

Oil and gas exploration in Uganda has raised concerns by the local people across the entire nation. This paper discusses the local people's knowledge, perceptions, and attitudes towards oil and gas operations in the protected area of Murchison Falls National Park. Data was collected between the months February and June 2019 from people living in and around the protected area. The study used a purposive sampling method to survey (n=50) participants. Participants included 10 Key Informants from Uganda Wildlife Authority, 10 oil and gas workers and 30 local people; 15 from Nwoya and 15 from Pakwach Districts. The views from the study included; possibility of damaged mammal habitats due to oil development, disruption of animal abundance and distribution, displacement of animals within the park as well as loss of biodiversity if the negative impacts are not mitigated. The perceptions of the local people



suggest as improvement in areas of oil waste management, noise, animal disturbance, and displacement. The local people expresses concerns that the protected area could stand a risk of losing its biodiversity due to further oil operations such as drilling.

KEYWORDS: Oil and Gas; Perceptions; Local Communities; Protected area; Uganda

Introduction

Uganda's initial evaluation of its hydrocarbon potential was conducted by E. J. Wayland, a government geologist, in the 1920s. He recorded multiple hydrocarbon occurrences in the Albertine Graben, with intermittent oil exploration continuing throughout the 1930s, with several shallow wells drilled around Butiaba and Kibiro. The deepest well, Waki-B-1, was drilled in 1938. Exploration activities halted due to World War II and post-independence turmoil. Serious exploration resumed in the early 1980s, starting with aeromagnetic data acquisition across the Graben, followed by ground geophysical and geological work in the late 1980s and 1990s. Seismic data collection began in 1998, with numerous surveys conducted since. Over one hundred wells have been drilled from 2002 onwards, with more planned. Uganda has made 21 oil discoveries in regions including Waraga, Kajubilirizi (Kingfisher), Nzizi, Ngassa, Taitai, Karuka, Ngege, Kasamene, Kigogole, Ngiri, Jobi-Rii, Nsoga, Wahrindi, Ngara, Nsoga, Mpyo, Jobi-East, Gunya, and Lyece.

In December 2005, the Mputa-1 well was drilled, striking oil on January 6, 2006, marking Uganda's first commercial oil discovery. According to the Petroleum Authority of Uganda, over thirty seismic surveys have been conducted, resulting in 7,254.6 line kilometers of 2D seismic data and 1,948.6 square kilometers of 3D seismic data. A total of 121 exploration and appraisal wells have been drilled, with 106 successfully discovering oil and/or gas, resulting in an impressive success rate of over 85%. This encompasses 39 exploration wells and 82 appraisal wells, with 36 of these wells having undergone flow testing.

While the environmental impacts of oil and gas operations in protected areas like national parks, particularly on biodiversity, receive considerable attention, the socio-environmental impacts and opportunity-threat dynamics associated with rapid energy development are often overlooked (Gramling and Freudenburg, 1992). Understanding local community perceptions is crucial for the success of any development project. Engaging with communities can foster open dialogue, influence decision-making, build trust, address community concerns, manage expectations, leverage local knowledge, and negotiate sustainable, locally relevant outcomes (Franks et al., 2012). Shepherd and Bowler (1997) argue that public perception significantly influences the acceptability of risks, beyond scientific criteria.

Recent sociological research highlights both positive and negative community impacts from unconventional shale gas development (Anderson and Theodori, 2009; Brasier et al., 2011; Weigle, 2011; Wynveen, 2011). In the Barnett Shale region, Theodori (2009) found that residents generally perceived social and environmental issues, such as truck traffic and freshwater use, as worsening due to development. Anderson and Theodori (2009) reported similar concerns among local leaders, including threats to public health, safety, environmental issues, and quality of life.



In 2012, the World Wildlife Fund (WWF) reported that Total E&P planned a nine-month seismic study of Murchison Falls National Park. In November 2018, the National Environment Management Authority (NEMA) organized two public hearings concerning the Environment and Social Impact Assessment (ESIA) Report for the Tilenga Oil Project. These hearings took place in the Bullisa and Nwoya districts and were conducted in collaboration with the Petroleum Authority of Uganda. Haselip and Romera (2011) noted the micro-level impacts of rapid oil and gas development on local communities and the environment. This study aims to understand the social perceptions of residents living in and around Murchison Falls National Park regarding oil and gas operations in the protected area.

Materials and Methods

Study Area

The study survey was conducted about related knowledge and perceptions of local people living in and adjacent to Murchison Falls National Park. Participants were selected from inside the protected area, Pakwach and Nwoya local communities. Murchison Park in Uganda is the country's biggest and most ancient conservation region, home to 76 types of mammals and 451 varieties of birds. It is found in the northwest region of Uganda, extending from the shores of Lake Albert inland, encompassing the area around the Victoria Nile and reaching up to the Karuma Falls. The park occurs within the Albertine Graben and it is managed by Uganda Wildlife Authority. The area was initially designated as a wildlife reserve in approximately 1934, and it subsequently transitioned into one of Uganda's earliest national parks in 1952. It is approximately 3,893 square kilometers in size, bisected by the Victoria Nile from east to west over a distance of about 115 kilometers. The park spans the Uganda districts of Buliisa, Nwoya, Kiryandongo, and Masindi.

Research Design

We used a survey by conducting key informant interviews as well as administering questionnaires. The study participants were selected on basis of their level of expertise in the field of oil and gas, their position in the administration ladder of oil and gas, availability and willingness to take part in the study. Fifty questionnaires (n=50) were administered to respondents between the month of February 2019 to June 2019. The research employed the purposive sampling technique to assemble the research study's population sample. This non-probability sampling method involved selecting sample members based on their familiarity, connections, and proficiency in the research subject (Freedman et al., 2007).

Research Ethical Considerations

During the research survey, we adhered to social research ethics by obtaining a research permit from the Uganda Wildlife Authority. Participation in the survey was entirely voluntary, and respondents had the right to withdraw from the study at any point. We provided a consent form outlining the study's details, allowing individuals to fully understand the implications of their participation and make an informed decision without any pressure or coercion. All collected data was kept confidential, and participants' permission was obtained to use their real names in the research. We ensured the dignity and well-being of participants were protected at all times, and we avoided using offensive, discriminatory, or otherwise unacceptable language in the formulation of the questionnaire.



Data Collection Tools and Methods

The key informants included 10 MFNP administrators, 10 Oil and Gas workers in the park and 30 local people from Pakwach and Nwoya Districts. The study used participants that had knowledge about the park before, during and after oil and gas exploration. The questionnaire was in English language and it contained structured, closed ended and open-ended questions in three sections; i.e. A, B and C. Section A investigated respondents demographics, Section B investigated the perception of oil and gas operations in the park and Section C investigated the possibility of restoration of mammal habitats after oil and gas operations, how it will be accomplished, the period of time it might take to restore a site to its original state, the techniques needed to restore the affected sites as well as major issues to be considered as part of conserving large mammals and their habitats amidst oil and gas operations.

Data Analysis

In creating the measurement for the constructs of this study survey, we primarily designed custom scales to fit the unique needs of this study, based on related conceptual research. All comments collected from the administered research questionnaires were coded and presented in themes and sub-themes. The data was demonstrated in form of bar graphs and later analyzed using chi-square tests to determine the significant differences in the responses given by the survey participants. In scenarios of incomplete returned responses, the data from those responses were excluded from the analysis.

Results

Compliance standards of oil operations in MFNP

The survey showed that 64% of the respondents (n=50) believe oil operations in the park meet the regulatory compliance standards. The number of respondents who agreed that oil operations in the park meet compliance standards were significantly different from the number of respondents that disagreed ($\chi^2=3.92$, d.f =1, $P<0.05$). The survey indicated that most respondents (90%) think that oil waste management is being done in a satisfactory manner. Similarly, most respondents (70%) indicated that there is no animal displacement and disturbance due to oil operations in the park (Figure 1). The number of respondents who are dissatisfied with oil waste management have a significant difference from the number of respondents that disagree ($\chi^2=32$, d.f =1, $P<0.05$). The number of respondents who feel that animal displacement and disturbance are a result of oil operations in the park are significantly different from the number of respondents that disagree ($\chi^2=8$, d.f=1, $P<0.05$).

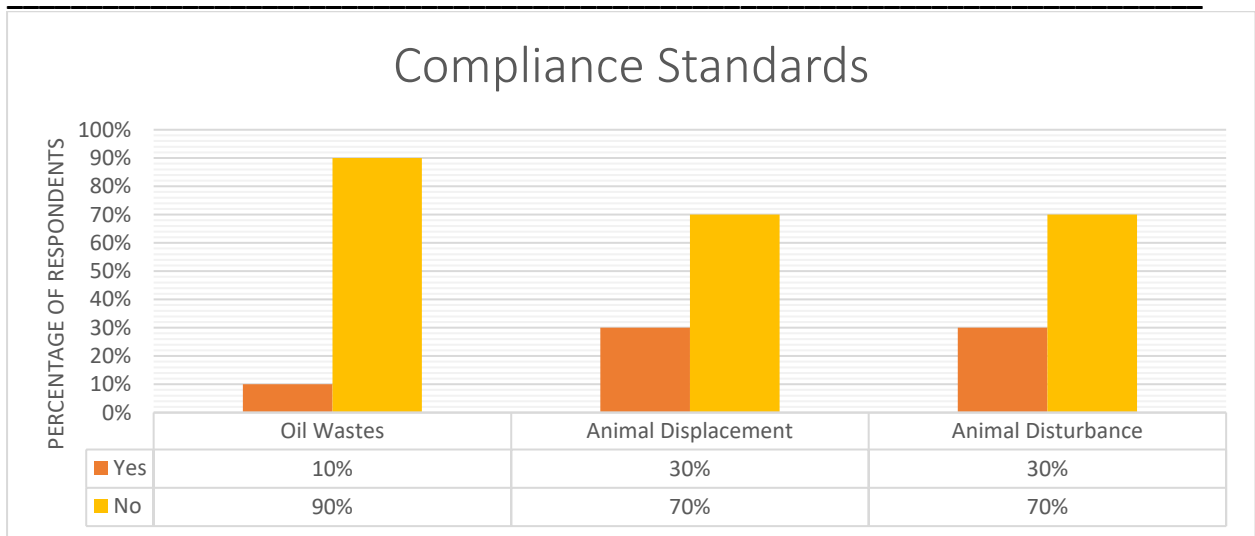


Figure 3: Percentage of dissatisfied compliance standards as suggested by respondents

Effects of oil operations on mammals

The results of the study show that 50% of respondents (n=50) feel that oil and gas operations in the park adversely affect mammals and their habitats. We further asked, in what ways the oil and gas operations were affecting mammals and their habitats in the protected area. The survey shows that; 32% of the number of respondents feel oil and gas operations cause noise, 44% of the number of respondents said it causes animal disturbance while 20% of the number of respondents(n=50) think it causes animal displacement (Figure 2). The number of respondents who think oil and gas operations causes noise are significantly different from the number of respondents that disagree ($\chi^2=3.84$, d.f=1, $P<0.05$). The number of respondents who suggested that animal disturbance is as a result of oil operations do not differ significantly from the number of respondents that disagree ($\chi^2=0.75$, d.f =1, $P>0.05$) and the number of respondents who think animal displacement is caused by oil operations in the park differ significantly from the number of respondents that disagree ($\chi^2=18$, d.f =2, $P<0.05$).

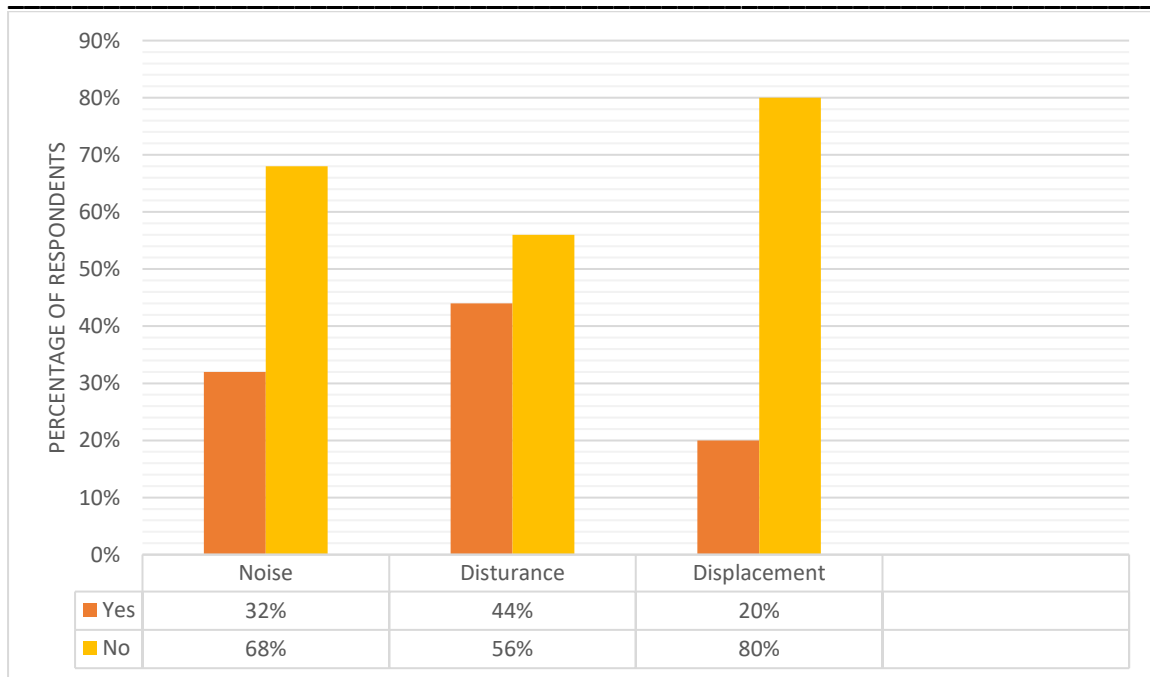


Figure 4: Percentage of oil and gas effects on mammals as suggested by respondents

Decommissioning effects of oil operations on Mammals

The decommissioning of an onshore oil pad entails the removal of all surface equipment, production tubing, and the uncasing of the well. To plug the well, specific wellbore sections are filled with concrete to stop the flow of reservoir fluids between different sections and to the surface. Following this, the surface casing is cut off, and a cap is welded several feet below ground, adhering to regulatory standards. The survey shows that 78% of the respondents (n=50) feel decommissioning of an oil well has effects on mammals. The number of respondents who think decommissioning of an oil pad causes effects on mammals differ significantly from the number of respondents who disagree ($\chi^2=15.68$, d.f=1, $P<0.05$). The study shows 62% of the respondents feel decommissioning of an oil pad causes animal displacement and 68% of the of respondents feel it causes relocation of animals (Figure 3). The number of respondents who think animal displacement is as a result of oil pad decommissioning are significantly different from the number of respondents that disagree ($\chi^2=2.88$, d.f=1, $P<0.05$). The number of respondents who suggested animal relocation is a result of decommissioning of an oil pad differ significantly from the number of respondents that disagree ($\chi^2=6.48$, d.f=1, $P<0.05$).

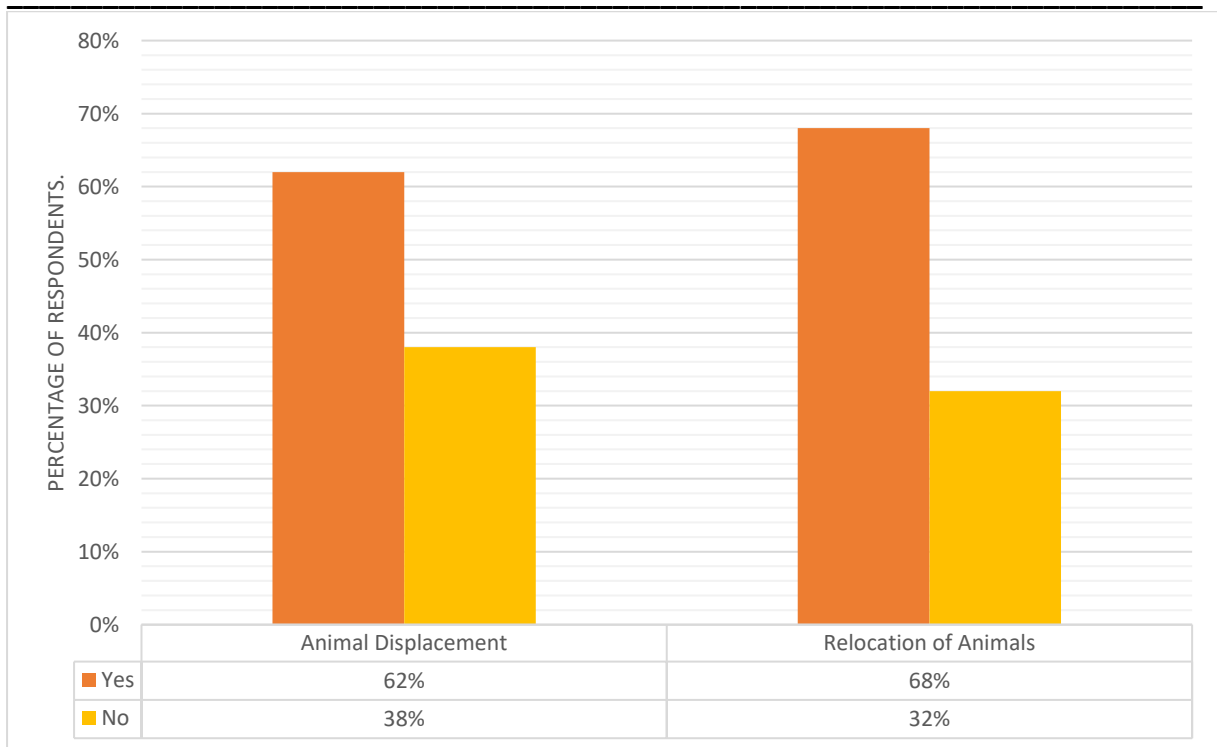


Figure 5: Percentage of decommissioning effects on mammals as suggested by respondents

Oil exploration in the protected area of Murchison Falls National Park

The survey shows 14% of the respondents (n=50) feel that oil operations in the park is a bad idea, 74% of the respondents feel the park is at risk of losing its biodiversity and 10% of the number of respondents think that under proper care it can co-exist with wildlife (Figure 4). The number of respondents who feel oil activities in the park are a bad idea are statistically significant from the number of respondents that disagree ($\chi^2=25.92$, d.f =1, $P<0.05$). The number of respondents who think that the park is at a risk of losing its biodiversity differ significantly from the number of respondents that disagree ($\chi^2=11.52$, d.f=2, $P<0.05$). The number of respondents who feel oil operations are a good idea under proper care differ significantly from the number of respondents that disagree ($\chi^2=32$, d.f=1, $P<0.05$).

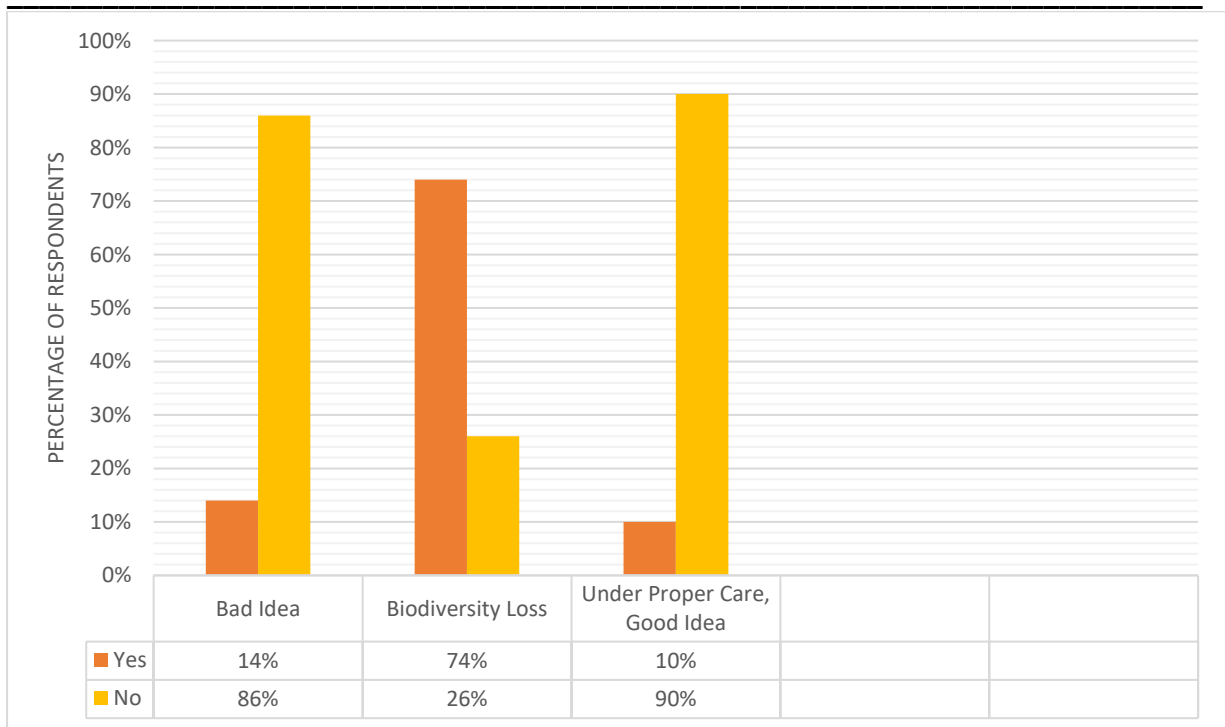


Figure 6: Percentage number of respondents' perception of oil and gas operations in the protected area

Restoration

Restoration is regarded as the final phase of an oil and gas project. When the production cycle of a field concludes and all extractable oil and gas have been processed, the facilities must be dismantled, and the surrounding area restored to its natural state. Respondents were asked whether it was possible to restore mammalian habitats to their natural state after oil and gas exploration and 52% of the respondents think it's possible. There is no significant difference in the number of respondents who agree and disagree that restoration of an oil pad is possible ($\chi^2=0.08$, d.f=1, $P>0.05$). When it was asked the period of time it would take for an oil pad to be restored to its natural state; 58% of the number of respondents feel it can take two years to restore an oil pad and 34% think it may take four years (Figure 5).The number of respondents who think two years is enough to restore an oil pad have no significant difference from the number of respondents that disagree($\chi^2=1.28$, d.f=1, $P>0.05$). The number of respondents that think four years is enough to restore an oil pad have a significant difference from the number of respondents that disagree ($\chi^2=5.12$, d.f =1, $P<0.05$).

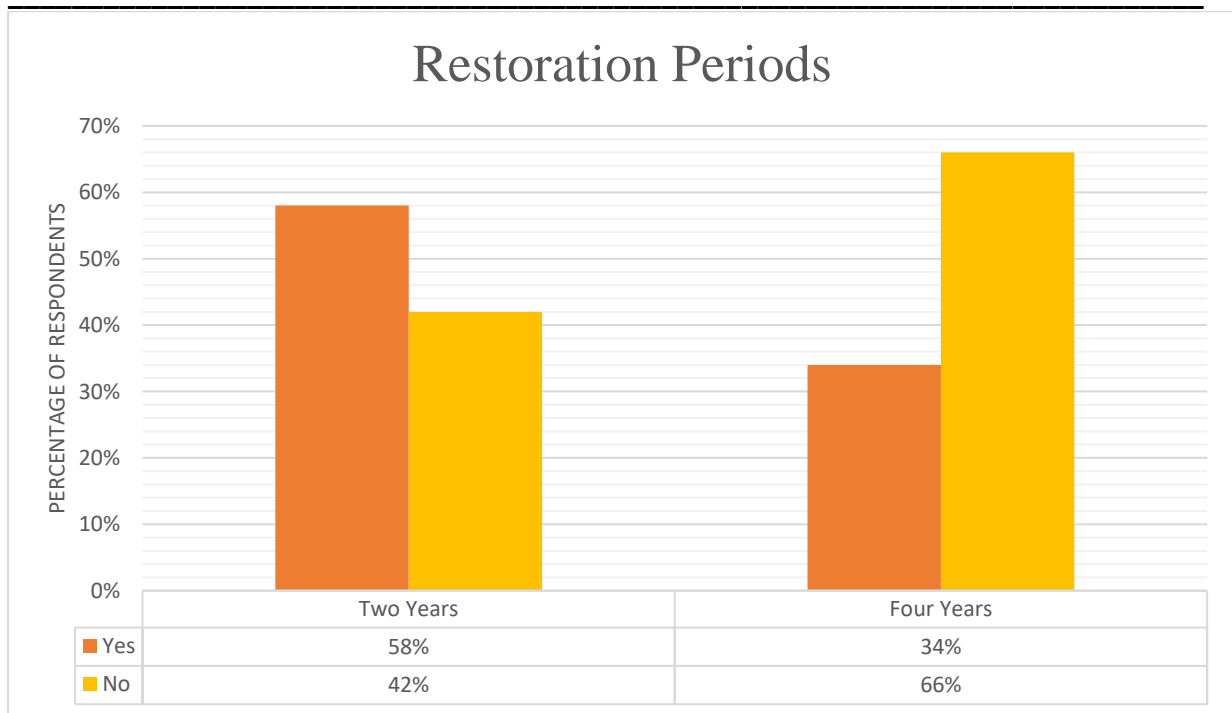


Figure 7: Percentage number of respondents' suggestion of the time period of oil pad restoration

Restoration techniques

The survey shows 96% of the respondents feel drainage ditch and excavation filling, and restoration of buffers are some of the techniques for restoring an oil pad/site and 100% of the respondents feel replanting of vegetation as the best technique to restore an oil pad back to its original state. The number of respondents who think filling drainage ditches, excavation fill, restoration of buffers can restore an oil pad differ significantly from the number of respondents that disagree ($\chi^2=42.32$, d.f=1, $P<0.05$). The number of respondents who suggested replanting of vegetation to restore an oil pad differ significantly from the number of respondents that disagree ($\chi^2=50$, d.f= 1, $P<0.05$).

Conservation of mammals and their habitats

The respondents were asked about the major issues that should be considered as part of the conservation of mammals and their habitats during oil and gas operations in the protected area. The survey shows 82% of the respondents feel core ecosystem parts such as cobleks should be conserved and oil operations should comply with EIA. 80% of the number of respondents feel that UWA guidelines of operating in the park should be upheld, 90% of the respondents feel oil and gas operations in the park should be monitored by the government, and 86% feel that in cases of oil operations, animals should be relocated to less disturbed areas. The number of respondents who think core ecosystem parts should be conserved and EIA compliance have a significant difference from the number of respondents that disagree ($\chi^2= 20.48$, d.f=1, $P<0.05$). The number of respondents that feel UWA guidelines should be upheld differ significantly from the number of respondents that disagree ($\chi^2=18$, d.f=1, $P<0.05$). There is a significant



difference between the number of respondents who agree and disagree with government monitoring of oil and gas operations in the park ($\chi^2= 35$, d.f=1, $P<0.05$). The number of respondents who suggested relocation of mammals differ significantly from the number of respondents that disagree ($\chi^2=25.92$, d.f=1, $P<0.05$).

Discussion

The study survey revealed that most residents believe oil operations in the park adhere to regulatory standards. However, they emphasized the need for better oil waste management and minimizing animal displacement and disturbance. This aligns with the UWA strategic plan, which acknowledges that oil exploration has led to habitat degradation, wildlife disturbances, and environmental damage (UWA, 2013). It is important to note that these findings do not suggest that oil companies in the park are failing to comply with regulatory standards for operations in protected areas; instead, they reflect the perceptions of key informants who responded to the survey.

The results indicate that decommissioning an oil pad after exploration impacts mammals by causing displacement and relocation. This view is consistent with O'Rourke and Connolly (2003), who argue that exploration, drilling, and extraction activities, whether onshore or offshore, are inherently disruptive and affect ecosystems, human health, and local cultures. However, these findings should not be seen as conclusive, as they are based solely on participant perceptions and lack scientific evidence.

The study also found that local residents fear the park's biodiversity will be compromised due to oil operations. This concern mirrors Wiegler's (2011) research, which identified resident concerns about impacts on key habitats, animal reproduction and migration, hunting, bird watching, and threatened/endangered species. Additionally, many respondents believe it is possible to restore oil pads to their natural state within two to four years, a finding consistent with Fuda's (2015) camera-trap study in the MFPA post-appraisal period, which found no evidence that wildlife avoided restored drill pads. Preliminary research by Plumtre et al. (2015) also suggests that while oil exploration affects animal behavior, animals return to these locations once the drill pads are restored.

Overall, the research survey highlighted increasing concerns and debates about unsatisfactory compliance standards, such as oil waste management and animal displacement and disturbance. This is in line with Pierson (2009), who found that residents voiced concerns about noise from drilling rigs and truck traffic, as well as concerns about the high noise levels from large compressor stations used to pump gas from well sites and public opinions of environmental damage (Brasier et al., 2011; Forsyth et al., 2007).

One of the primary concerns identified in the study was the restoration of oil sites after exploration. The results indicated that restoration is feasible through various techniques, such as filling drainage ditches, excavation fill, restoration buffers, and replanting vegetation. Given the inevitable land degradation during exploration preparations, understanding vegetative restoration processes is essential (Winslow et al., 2009). If wildlife habitats and ecological degradation are not addressed through effective reclamation practices, future natural resource development could be constrained (Stahl and Williams, 2010). Participants suggested replanting vegetation as a restoration technique, which aligns with McKendrick's (1997)



assertion that reclamation projects should establish plants quickly and allow natural succession to occur, eventually leading to vegetation similar to the surrounding tundra. Another recommended technique was filling excavation and drainage ditches, similar to McKendrick et al.'s (1992) approach, which involves identifying native plant species, testing various seeding methods, and adjusting ground fill to improve plant establishment.

Conclusions and Recommendations

This study survey results show that respondents believe oil and gas operations meet the set compliance standards of operating in the protected area of Murchison Falls National Park. However, oil waste management, animal displacement and disturbance arising from oil and gas operations can be improved. The survey shows that people feel oil and gas activities affect mammals through noise, disturbance and displacement. The study respondents suggested that decommissioning of an oil pad affects mammals through displacement thus leading to the relocation of animals from their habitats. The study respondents think that oil and gas exploration in the protected area is a bad idea and the park is at risk of losing its biodiversity. However, some respondents believe that it can co-exist alongside wildlife if proper care/precautions are taken. The respondents from the research survey think there is a possibility of restoring mammal habitats once oil and gas exploration has been completed in a minimum of two years up to a maximum of four years. The possible restoration techniques suggested by participants included; filling drainage ditches, excavating fill, restoration buffers and replanting of vegetation. Respondents feel that amidst oil and gas operations in the protected area, core ecosystem parts should not be tampered with, ensure ESIA compliance; uphold UWA guidelines of operating in protected areas and re-allocation of animals to less disturbed areas of the park if possible.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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