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***In Vitro* Antimicrobial Activity of Crude Extracts of *Erythrina abyssinica* and *Capsicum annum* in Poultry Diseases Control in the South Western Agro-Ecological Zone of Uganda**

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1. Introduction

Many small holder farmers in the south western agro-ecological zone (SWAEZ) of Uganda have for a very long time been using medicinal plants especially *Erythrina abyssinica* and *Capsicum annum* for the management of worms, Newcastle disease and other microbial infections respectively in local poultry (Nsubuga-Mutaka *et al.*, 2005 and Lagu and Kayanja (2010). Commonly the root barks of *Erythrina abyssinica* are picked crushed and mixed with water and administered to the birds. The ripped fruits of *Capsicum annum* are picked crushed and mixed with solutions of ash and water (ITDG and IIRR) (1996); Lagu and Kayanja (2010). These medicinal combinations are mainly given to birds to treat them against worms and other microbial infections and worms (ITDG and IIRR, 1996). The farmers have been using these medicinal plant extracts for a very long time Katunguka-Rwakishaya *et al.*, 2004; Olila *et al.*, 2007; Ejobi *et al.*, (2007). It is however, not clear if the *Erythrina abyssinica* and *Capsicum annum* have activity against the common microbes that affect the poultry. This study aims to investigate the anti-microbial activities and minimum inhibitory concentrations (MICs) of *Erythrina abyssinica* and *Capsicum annum* used by the farmers in the control and treatment of common poultry infections in the south western agro-ecological zone of Uganda.

2. Materials and methods

2.1 Identification and description of medicinal plants (for *Erythrina abyssinica* and *Capsicum annum*)

2.1.1 *Erythrina abyssinica* (Leguminosae)

Erythrina abyssinica (Leguminosae) is a species of leguminous tree as seen in Figure 1. It is distributed in Congo Republic (ex Belgian), the Sudanese Republic, Ethiopia, Eritrea,

Uganda, Kenya, Tanzania and Zimbabwe. In northern and western Ethiopia, it is found at elevations between 1600 and 2100 m. *Erythrina abyssinica* (Luganda name: Muyirigiti or Jjirikiti; Runyakole name: Ekiko), is a deciduous savannah legume. It grows in open woodland and grassland. It has characteristic red overflowing flowers. It can be propagated through seedlings, cuttings and truncheons. In the south western rangelands of Uganda, it is sometimes planted along fences of paddocks to support barbed wires. It has various traditional medicinal applications in livestock. It is also used in traditional human medicine Figure 2.



Fig. 1. *Erythrina abyssinica* tree



Fig. 2. Dry root barks of *Erythrina abyssinica*

2.1.2 *Capsicum annum* (*solanaceae*)

Capsicum annum (*solanaceae*) was identified in the study areas by a botanist from Mbarara University of Science and Technology. *Capsicum annum* belongs to the kingdom plantae plants, subkingdom of tracheobiota (vascular plants), super-division of spermatophyta (seed plants), division of magnoliophyta (flowering plants), class magnoliopsida (dicotyledons), subclass, asteridae, order, solanels, family of solanaceae (potato family), genus capsicum L. (pepper), species *Capsicum annum* L. (cavenne pepper) and variety *Capsicum annum* L. var *annum* (Cayenne pepper).

Capsicum annum is a perennial shrub growing up to 2 m (6') in height, with woody a trunk? Its leaves have various shapes usually elliptical up to 10 cm (4") long as seen in Figure 1. The flowers are white to yellowish in groups of 2 or 3 followed by small, upright, fiery, green fruits that ripen to red. The active ingredient in the plant is *capsaicin* that is used for management of various medical conditions. The varieties of this "fruit" vary greatly in size, color and pungency. The plant extract that provides therapeutic action is the seed oil.

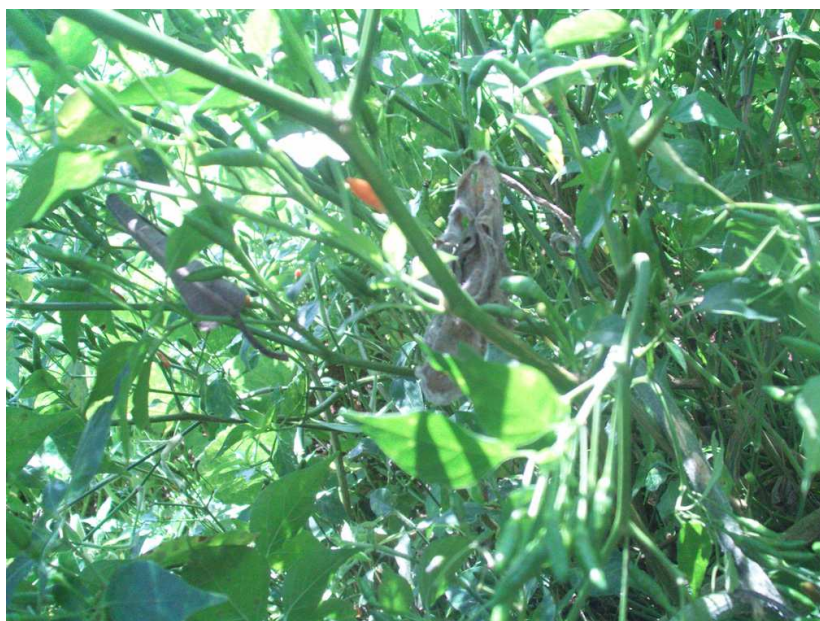


Fig. 3. *Capsicum annum* plant

2.2 Sample collection and post harvest handling (for *Erythrina abyssinica* and *Capsicum annum*)

Information gathered included vernacular names and parts used in the preparation of herbal remedies. The plants were identified by botanist from Mbarara University of Science and Technology. Voucher specimens were deposited in the University. Fresh samples of the plant materials (root barks, stem barks and leaves) of *Erythrina abyssinica* were collected from 500m away from Rubare town in Ntungamo district on 19th May, 2010. Four (4) km away from Rubindi sub county in Mbarara district on 22nd May, 2010. Seven (7) km away from Bugongi sub county in Bushenyi district on 23rd May, 2010 and 4 km away from Lwanda Sub County GPS location S00040.379'; E031028.7779, Rakai district on 16th June, 2010. The seeds and leaves of *Capsicum annum* were collected during early morning to late afternoon and placed in a plastic bag and stored in the vehicle

During field collection of the samples, the sites where the plants were found were geographically and ecologically described. Ejobi *et al.*, 2007 found out that these plants were abundantly found in all areas in the SWAEZ. The quantity of each plant biomass collected depended on approximately with the amount of plant biomass needed to yield enough concentrates for in vitro studies. The plant materials were kept in a plant press in the department of Biology, Mbarara University of Science and Technology. The plants were dried at room temperature at Mbarara Zonal Agricultural Research and Development Institute (Mba ZARDI). The partially dried plants samples were collected and packed in a plastic bag and then transported to Natural Chemotherapeutics Research laboratories (NCRL) Wandegaya, Kampala for extraction and concentration of plant extracts.

The concentrated samples were taken to the Microbiology and Parasitology Laboratory of the School of Veterinary Medicine, Makerere University for the activity studies. Voucher specimens of the plants collected were as follows; Root barks Ntu001Ea, Bus 002Ea, Mbra 003 Ea, Rakai 004 Ea; the stem barks included Ntu005Ea, Bus 006Ea, Mbra 007Ea, Rakai 008 Ea. The leaves sample include Ntu009Ea, Bus 010Ea, Mbra 011Ea, Rakai 012 Ea.

The Voucher specimens for *Capsicum annum* viz; Leaves Ntu001Ca, Bus002Ca, Mbra003Ca, Rakai 004Ca. Fruits Ntu005Ca, Bus006Ca, Mbra007Ca, Rakai008Ca of the plants studied was kept in the departmental laboratory.

Drying and milling

The leaves, root bark and stem bark of *Erythrina abyssinica* and the leaves and fruits of *Capsicum annum* from Mbarara, Bushenyi, Ntungamo and Rakai districts were dried at 50-60°C in a vacuum oven for 24 hours. The dry plant material samples were milled using an electric grinder in fine particles.

2.3 Obtaining process of crude extracts of the plant parts used, including the determination of 'extract yield' and "Standardization of dosages"

The leaves, root bark and stem bark of *Erythrina abyssinica* leaves and fruits of *Capsicum annum*

2.3.1 Extraction, filtration and concentration

The milled samples (100-500g) were soaked in 70% ethanol (5L) for 48hours with frequent shaking. Thereafter the extracts were filtered first with cotton wool followed by Whatman filter paper® and stored in at room temperature. The filtrate was then concentrated using vacuum rotary evaporator. The concentrates were then dried in vacuum oven at 60°C to dryness.

2.3.2 Determination of extract yield

The percentage yield of the extract was determined gravimetrically using the dry weight of extract (x) and soaked samples material (y) as follows

$$\text{Percentage yield} = x/y * 100 \quad (1)$$

2.3.3 Standardization of dosages

The information gained on the percent yields of crude extracts was used for standardizing dosage rates of fine powder preparations of the plant materials. For example, the amount of crude extract contained in a known weight of fine powder of plant materials was calculated from the formulae given above.

2.4 Determination of antimicrobial activity of the leaves, root bark and stem bark of *Erythrina abyssinica*, the leaves and fruits of *Capsicum annum*

The concentrated extracts of the roots, stem and leaves of *Erythrina abyssinica* and *Capsicum annum*, from the National Chemotherapeutics Laboratory, Wandegaya were transported to the department of microbiology laboratory at the School of Veterinary Medicine, Makerere University.

2.4.1 Materials required antimicrobial activity

Bacterial culture; Mueller Hinton agar medium; Nutrient broth; Plant extracts; Bunsen flame; Micro pipettor (10 μ l-200 μ l), adjustable; Spreader/wire loop; Agar borer and Incubator.

2.4.2 Method antimicrobial activity

Approximately 2-5 freshly grown bacterial colonies of the test organism were emulsified into Nutrient broth and incubated for 10-15 minutes at room temperature. With a spreader of wire loop, Mueller Hinton agar plate was evenly inoculated and plate allowed to stand for 5 minutes at room temperature (i.e. 25°C).

Using a sterile agar borer (Sterilized using a bunsen flame), wells were dug into the inoculated agar at reasonable distance apart (approximately 5 cm).

The plant extract(s) were transferred into the created agar wells till when full. Extract were not allowed to float on the agar surface. The plate lid was replaced and did not turn the petri-dish upside down. The setup was incubated at 37°C overnight. The presence for bacterial inhibition zones around each well looked for. Appearance of clear zones around a well was indicative of the anti bacterial activity of an extracts (Bizimenyera *et al.*, 2005).

2.4.3 Determination of the Minimum Inhibitory Concentration (MIC) of extract

2.4.3.1 Materials for Minimum Inhibitory Concentration (MIC) of extract

Broth culture of test organism (s); known concentration of plant extracts (e.g. 0.5g/ml, 1g/ml etc); set of test tubes; micro-pipettor (adjustable 100 μ l-1000 μ l); Nutrient broth; Sterile physiological saline; incubator.

2.4.3.2 Method for Minimum Inhibitory Concentration (MIC) of extract

A set of test tubes were dispensed 0.5 ml (500 μ l) of physiological saline. An equal volume (i.e. 0.5ml) of test plant extract were added to the saline in first tube and mixed the two thoroughly well.

This was repeated throughout all the test tube and the last aliquot 0.5 ml of solution from the last tube discarded so as to have uniform volume. This constituted a two fold serial dilution whereby each step moved to the right reduced the concentration of the extract by a factor of 2. About 100 μ l of a 24 hour culture of the test organism were added to each of the test tubes (containing the already serially diluted extract). This was mixed thoroughly well, plugged with cotton wool and incubated the preparation at 37°C for 16-24 hours.

At microbiology laboratory, the samples were each weighed and dissolved in Dimethylsulfoxide (DMSO) at a final concentration of 0.5g/ml. Mueller Hinton Agar (MHA) plates for antibiotic sensitivity testing were prepared and inoculated with pure colonies of *E. coli*, *Staphylococcus spp.*, *Streptococcus spp.*, *Salmonella spp.*, and *Pseudomonas spp.*, which were known to be the common causes of poultry diseases.

The wells were bored in the inoculated plates and the samples from the extracts were impregnated into the wells and incubated overnight. A control plate inoculated with *E. coli* and impregnated with Dimethylsulfoxide (DMSO) and a known antibiotic, Ciprofloxacin was set up as negative and positive controls respectively.

After 24hrs of incubation, the plates were examined for antibacterial activity on the different sample extracts, and the results were as follows:

2.4.4 Observation/ interpretation of results

The inoculated test tubes were examined for inhibition of growth, where there is antibacterial activity; there was inhibition of growth, thus no turbidity in the test tube. The minimum inhibitory concentration (MIC) of a compound (extract) in this case the least concentration to have inhibited bacterial growth.

2.5 Data analysis

Data collected were entered in Excel windows 2007 (Microsoft Corporation). Frequencies, means and graphs were derived to explain phenomenon on significance and relationships to activities and minimum inhibitory concentrations of crude extracts of root, stem barks and leaves of *Erythrina abyssinica* and leaves and fruits of *Capsicum annum*.

2.6 Results

2.6.1 Crude extracts of root, stem barks and leaves of *Erythrina abyssinica*

It is clear from Figure 4 and Table 1 that root barks and stem barks of *Erythrina abyssinica* have better yields than the leaves.

In-vitro microbiological studies on *Erythrina abyssinica* indicate that root and stem bark extracts have activities against *Staphylococcus aureus* in Ntungamo, Mbarara, Bushenyi and Rakai. *Pseudomonas auroginosa* in Mbarara, Bushenyi except leaves from Bushenyi. No activities were noted on *E. coli* and *Salmonella species* as detailed in table 2 and Figure 5.

It has been demonstrated that root and stem barks have activity against *Staphylococcus aureus* in all districts except stem bark for Rakai. The root barks, stem barks and leaves of Mbarara,

Bushenyi respectively were effective against *Pseudomonas aeruginosa* demonstrated in Table 3. The study found that the leaves extracts did not have antimicrobial activities.

| Plant Sample | Location | Plant part | Weight of sample (g) | Dry weight of concentrate (g) | % yield |
|----------------------|----------|------------|----------------------|-------------------------------|---------|
| <i>E. abyssinica</i> | Ntungamo | Root barks | 450.0 | 39.6 | 8.8 |
| | Mbarara | Root barks | 295.6 | 37.8 | 12.8 |
| | Bushenyi | Root barks | 689.5 | 53.0 | 7.7 |
| | Rakai | Root barks | 500.0 | 24.3 | 4.9 |
| | Ntungamo | Stem barks | 537.0 | 11.6 | 2.2 |
| | Mbarara | Stem barks | 435.8 | 10.7 | 2.5 |
| | Bushenyi | Stem barks | 483.7 | 13.4 | 2.8 |
| | Rakai | Stem barks | 500.0 | 44.6 | 8.9 |
| | Ntungamo | Leaves | 500.1 | 3.2 | 0.6 |
| | Mbarara | Leaves | 451.7 | 3.2 | 0.7 |
| | Bushenyi | Leaves | 761.6 | 29.4 | 3.9 |
| | Rakai | Leaves | 500.0 | 31.1 | 6.2 |

Table 1. Percentage yield extract of leaves, root bark and stem bark of *Erythrina abyssinica* from Mbarara, Bushenyi, Ntungamo and Rakai districts

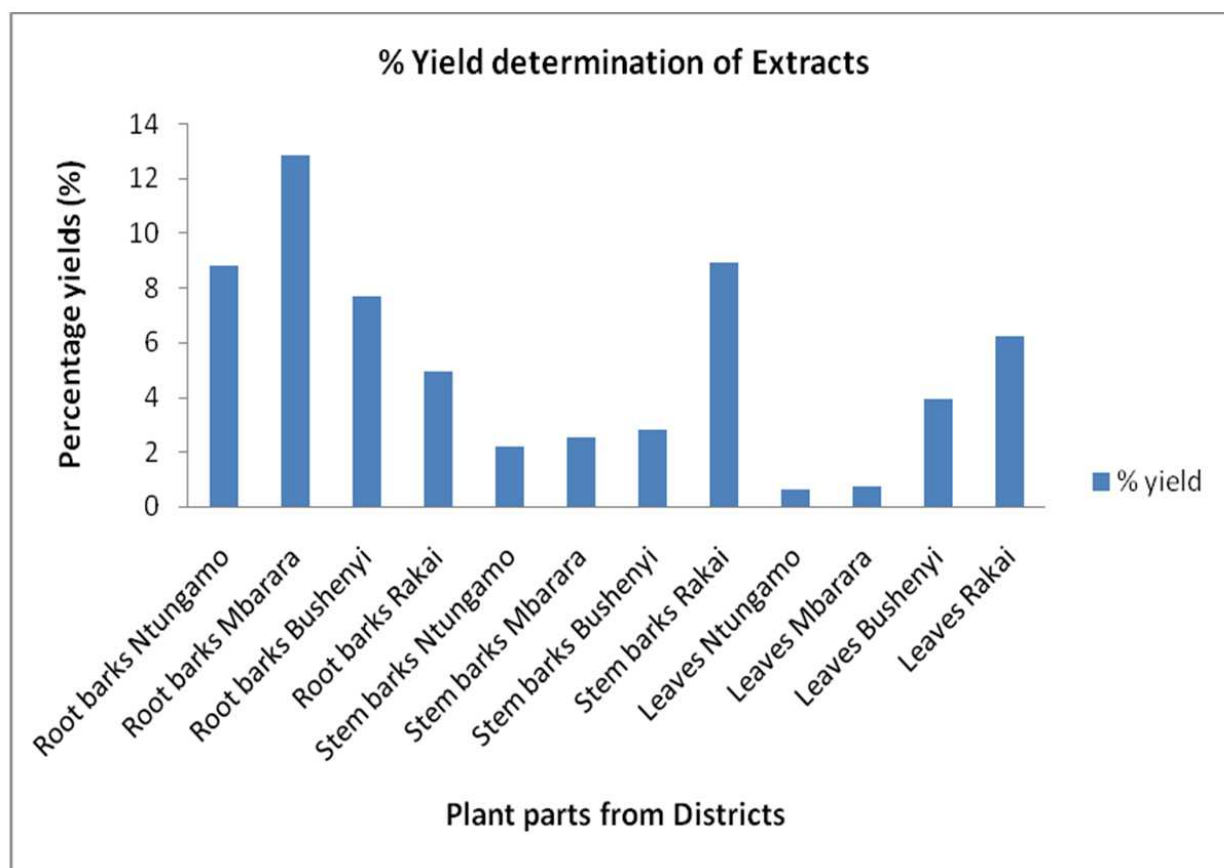


Fig. 4. Percentage yield determination of plant extracts (*Erythrinna abyssinica*)

Antimicrobial assay of plant extracts against selected bacteria as indicated below

| No. | Identity/ Name of Extracts | Inhibition zone (mm) | | | | District |
|-----|----------------------------|----------------------|-------------------|------------------------------|-------------------------------|----------|
| | | <i>E. coli</i> | <i>Salmonella</i> | <i>Staphylococcus aureus</i> | <i>Pseudomonas aeruginosa</i> | |
| 1 | Root bark Ea Ntungamo | 0 | 0 | 13 | 0 | Ntungamo |
| 2 | Root bark Ea Bushenyi | 0 | 0 | 14 | 0 | Bushenyi |
| 3 | Root bark Ea Mbarara | 0 | 0 | 12 | 8 | Mbarara |
| 4 | Root bark Ea Rakai | 0 | 0 | 15 | 0 | Rakai |
| 5 | Stem bark Ea Ntungamo | 0 | 0 | 11 | 0 | Ntungamo |
| 6 | Stem bark Ea Bushenyi | 0 | 0 | 12 | 7 | Bushenyi |
| 7 | Stem bark Ea Mbarara | 0 | 0 | 14 | 0 | Mbarara |
| 8 | Stem bark Ea Rakai | 0 | 0 | 0 | 0 | Rakai |
| 9 | Leaves Ea Ntungamo | 0 | 0 | 0 | 0 | Ntungamo |
| 10 | Leaves Ea Bushenyi | 0 | 0 | 0 | 8 | Bushenyi |
| 11 | Leaves Ea Mbarara | 0 | 0 | 0 | 0 | Mbarara |
| 12 | Leaves Ea Rakai | 0 | 0 | 0 | 0 | Rakai |

Table 2. Antimicrobial assay of *Erythrina abyssinica* plant extracts against selected bacteria

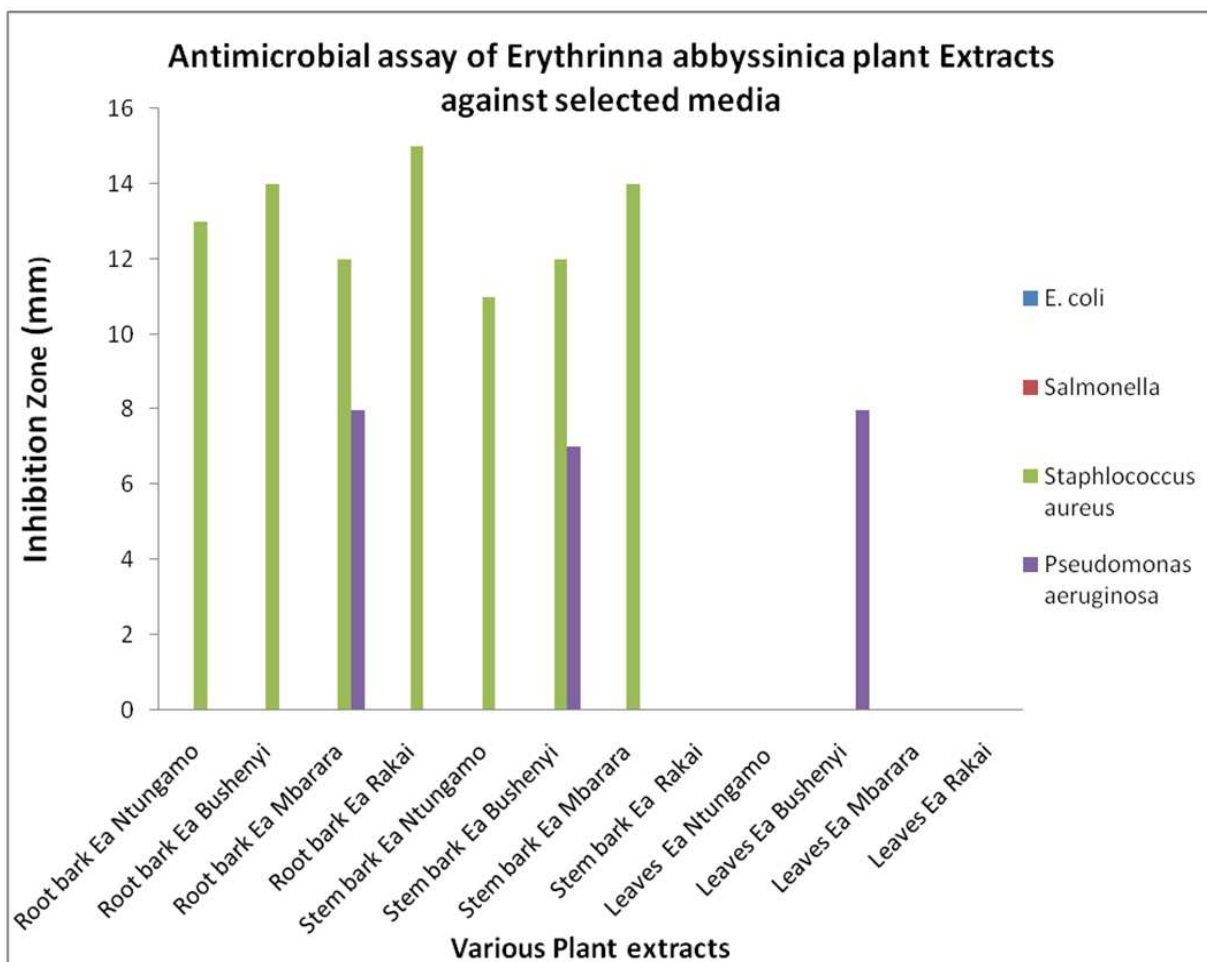


Fig. 5. Antimicrobial assay of plant extracts from four districts against selected media

| No. | Identity/ Name of Extracts | Minimum Inhibitory concentration of different micro-organisms (g/ml) | | | | |
|-----|----------------------------|--|----------------|-------------------|------------------------------|-------------------------------|
| | | Original sample concentration (g/ml) | <i>E. coli</i> | <i>Salmonella</i> | <i>Staphylococcus aureus</i> | <i>Pseudomonas aeruginosa</i> |
| 1 | Root bark Ea Ntungamo | 0.3 | 0 | 0 | 0.0047 | 0 |
| 2 | Root bark Ea Bushenyi | 1 | 0 | 0 | 0.0313 | 0 |
| 3 | Root bark Ea Mbarara | 1 | 0 | 0 | 0.0313 | 1 |
| 4 | Root bark Ea Rakai | 1 | 0 | 0 | 0.0156 | 0 |
| 5 | Stem bark Ea Ntungamo | 0.25 | 0 | 0 | 0.0039 | 0 |
| 6 | Stem bark Ea Bushenyi | 0.41 | 0 | 0 | 0.0256 | 0.41 |
| 7 | Stem bark Ea Mbarara | 0.224 | 0 | 0 | 0.0035 | 0 |
| 8 | Stem bark Ea Rakai | 1 | 0 | 0 | 0 | 0 |
| 9 | Leaves Ea Ntungamo | 0.39 | 0 | 0 | 0 | 0 |
| 10 | Leaves Ea Bushenyi | 1 | 0 | 0 | 0 | 1 |
| 11 | Leaves Ea Mbarara | 0.412 | 0 | 0 | 0 | 0 |
| 12 | Leaves Ea Rakai | 1 | 0 | 0 | 0 | 0 |

Table 3. Minimum Inhibitory concentration of different micro-organisms

2.6.2 Crude extracts of leaves and fruits of *Capsicum annum*

The yields of *Capsicum annum* leaves and fruits are detailed in Figure 4. It is clear from the tabular representation that leaves had better yield than fruits.

Studies on *Capsicum annum* indicate that fruits extracts of Mbarara have activities on *Salmonella* species and *Pseudomonas auroginosa* for the case of fruit extracts from Rakai. No microbial activities were noted against *Staphylococcus aureus* and *E.coli*. It was demonstrated that leaves extracts have no microbial activities as illustrated in Table 5 and Figure 7.

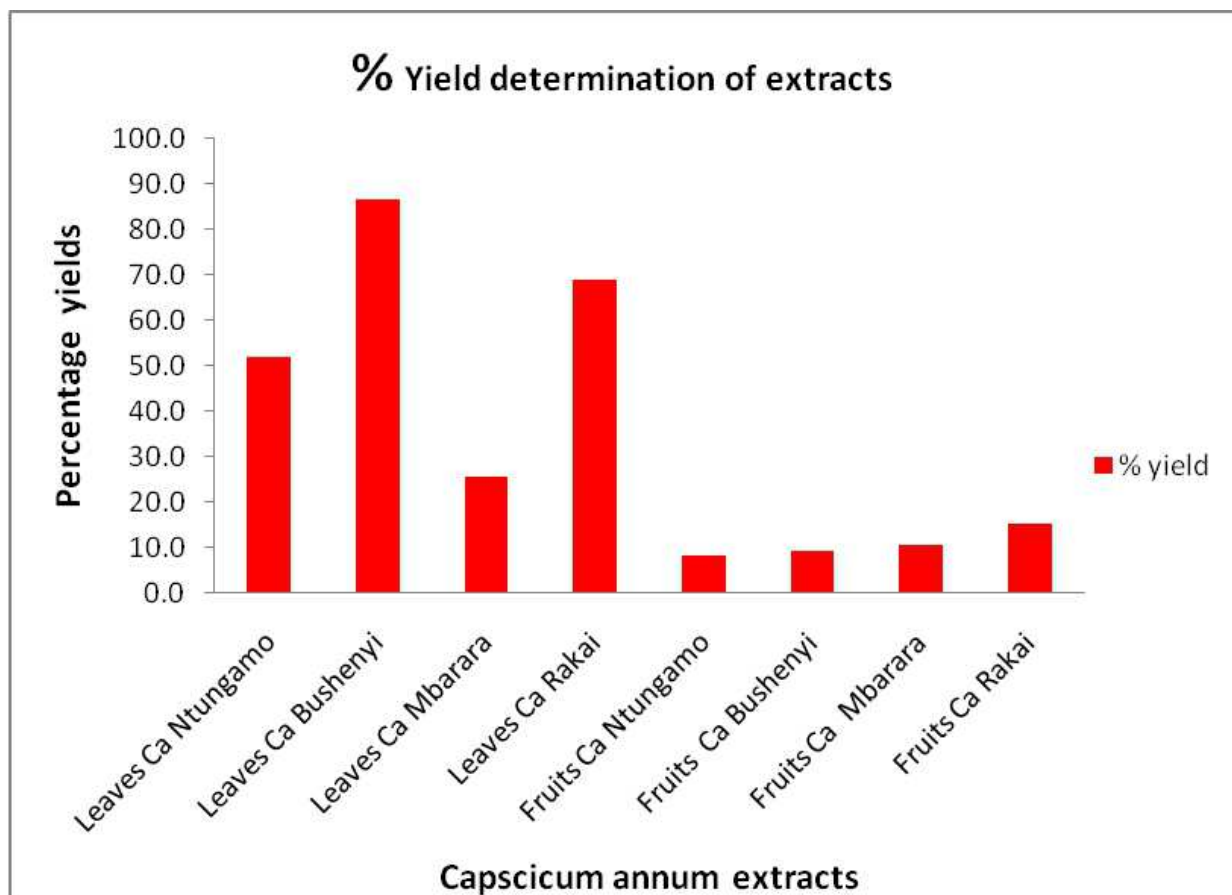


Fig. 6. Percentage yield determination for the plant *Capsicum annum*

Results indicated that leaves of *Capsicum annum* have better yields than fruits of *Capsicum annum* in all the four districts Mbarara, Ntungamo, Rakai and Bushenyi.

Antimicrobial assay of plant extracts against selected bacteria as indicated below

| No. | Identity/ Name of Extracts | Inhibition Zone (mm) | | | | District |
|-----|----------------------------|----------------------|-------------------|------------------------------|-------------------------------|----------|
| | | <i>E. coli</i> | <i>Salmonella</i> | <i>Staphylococcus aureus</i> | <i>Pseudomonas aeruginosa</i> | |
| 1 | Leaves Ca Ntungamo | 0 | 0 | 0 | 0 | Ntungamo |
| 2 | Leaves Ca Bushenyi | 0 | 0 | 0 | 0 | Bushenyi |
| 3 | Leaves Ca Mbarara | 0 | 0 | 0 | 0 | Mbarara |
| 4 | Leaves Ca Rakai | 0 | 0 | 0 | 0 | Rakai |
| 5 | Fruits Ca Ntungamo | 0 | 0 | 0 | 0 | Ntungamo |
| 6 | Fruits Ca Bushenyi | 0 | 0 | 0 | 0 | Bushenyi |
| 7 | Fruits Ca Mbarara | 0 | 8 | 0 | 0 | Mbarara |
| 8 | Fruits Ca Rakai | 0 | 0 | 0 | 7 | Rakai |

Table 5. Antimicrobial assay of *Capsicum annum* plant extracts against selected bacteria

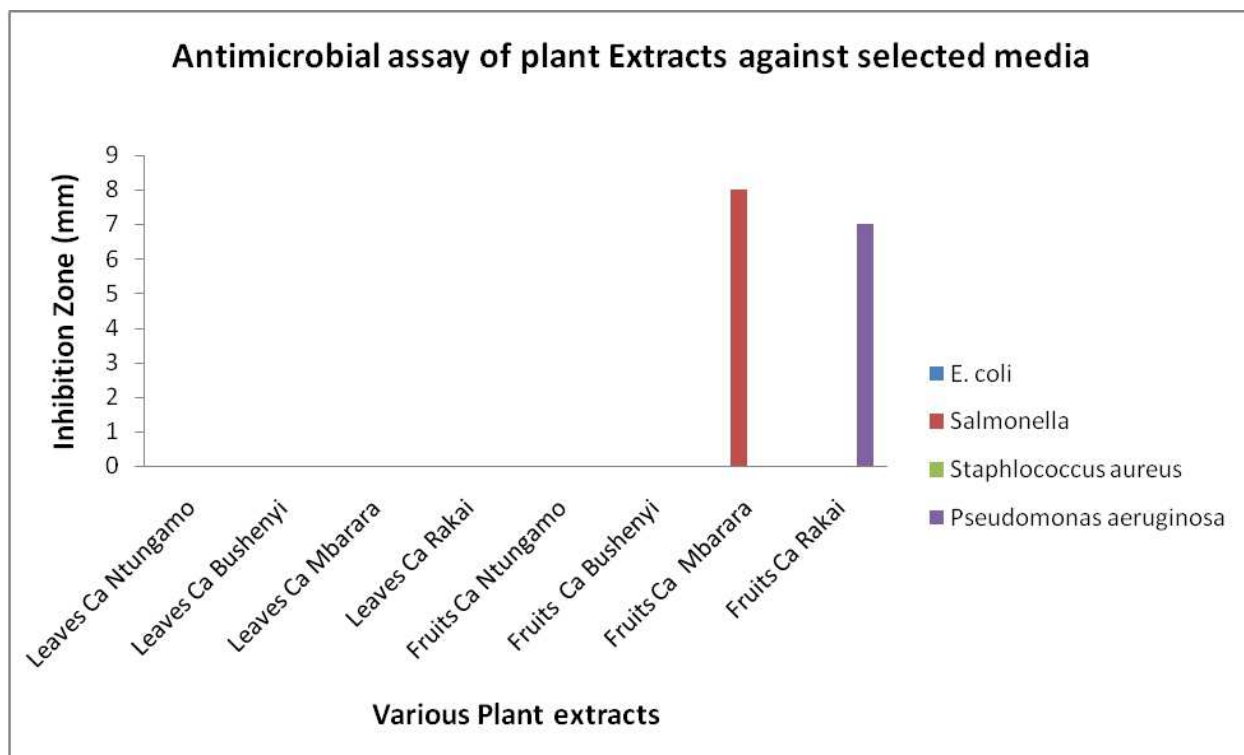


Fig. 7. Antimicrobial assay of plant extracts against selected bacteria.

It was demonstrated that root and stem barks had activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* demonstrated in Table 3.

| Sample ID | Original sample concentration (g/ml) | MICs of Extracts to different microorganisms (g/ml) | | | |
|--------------------|--------------------------------------|---|-------------------------------|-------------------|---------------|
| | | <i>Staphylococcus aureus</i> | <i>Pseudomonas aeruginosa</i> | <i>Salmonella</i> | <i>E.coli</i> |
| Leaves Ca Ntungamo | 1 | 0 | 0 | 0 | 0 |
| Leaves Ca Bushenyi | 1 | 0 | 0 | 0 | 0 |
| Leaves Ca Mbarara | 1 | 0 | 0 | 0 | 0 |
| Leaves Ca Rakai | 1 | 0 | 0 | 0 | 0 |
| Fruits Ca Ntungamo | 0.328 | 0 | 0 | 0 | 0 |
| Fruits Ca Bushenyi | 1 | 0 | 0 | 1 | 0 |
| Fruits Ca Mbarara | 1 | 0 | 0 | 0 | 0 |
| Fruits Ca Rakai | 1 | 0 | 1 | 0 | 0 |

Table 6. Minimum inhibitory concentration of different micro-organism

Fruits of *Capsicum annum* exhibited minimum inhibitory concentration against *Salmonella* species and *Pseudomonas aeruginosa* detailed in table 6.

2.7 Discussion

2.7.1 Crude extracts of root, stem barks and leaves of *Erythrina abyssinica*

Yields of the plants extracts

The results show that the yields of root barks perform better than the stem barks and leaf extracts detailed in Table 1 and Figure 3. There is an average 8.6%, 5.5% and 2.9% yield for the root barks, stem barks and leaves respectively in the districts of Ntungamo, Mbarara, Bushenyi and Rakai. The information gained on the percent yields of crude extracts were used for standardizing dosage rates of fine powder preparations of the plant materials. For example, the amount of crude extract contained in a known weight of fine powder of plant materials can then be calculated. This agrees with findings by (Ejobi F and Olila D 2004; Olila *et al.*, 2007).

Antibacterial activities of the plant extracts

The antimicrobial activity of crude extracts of root barks and stem barks of *Erythrina abyssinica* had activity against *Staphylococcus aureus* in Ntungamo, Bushenyi, Mbarara and Rakai districts except stem bark from Rakai. There was activity against *Pseudomonas aeruginosa* in Mbarara and Bushenyi respectively as detailed in table 2 and figure 4. Generally, leaves extracts showed no microbial activity except for leaf extracts from Bushenyi district. No antibacterial activities were noted against *Salmonella* species and *E.coli*.

The attributing factor for anti bacterial activity of the crude fruit extracts could be due to presence of bioactive constituents' in the extracts. This agrees with findings by Masola *et al.*, 2009 and Ogundare *et al.*, (2006).

Moriyasu *et al.*, (1998) collected stem bark *E.abyssinica* from Meru district of Kenya. They carried structural elucidation of new flavanones isolated from *E.abyssinica* and found presence of prenylated flavanones (abyssinin I(1), II (2), III(3), along with abyssinone V, sigmoidin A, B, C and F, and sigmoidin B 4'-(methyl ether). These compounds exhibited antimicrobial activities.

Further still, the presence of bioactive compounds viz; abyssinoflavanone IV, V and VI posses some antimicrobial activities as reported by (Ichimaru *et al.*, 1996).

The stem woods of *Erythrina latissima* another species of *Erythrina* have two isoflavones and a flavanone with isolates Isoflavone (erylatissin A, B, C) in addition to 10 known flavonoids. These compounds exhibited antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida mycoderma* (Chacha *et al.*, 2004). This study found that *Erythrina abyssinica* was effective against *Staphylococcus aureus* and *Pseudomonas aeuroginosa*.

The root and stem extracts of *Erythrina abyssinica* however did not show any microbial activity against *Salmonella* species and *Escherichia coli*.

A study by Masola *et al.*,(2009) in Mpwapwa, in the semi arid central zone of Tanzania found out that bioactive constituents of terpenoids, tannins, phlobotannins, saponins and cardiac glycosides were found to be present in the stem barks of the plant *Adansonia digitata* (Bombacaceae) (African baobab). It would therefore mean similar bioactive compounds were present in the plant *Erythrina abysinnica*.

Ikigai *et al.*, (1993) indicated that purified tannins, saponins and terpenoids have anti microbial activity. These bioactive compounds were reported to be effective against gram positive and gram negative bacteria. They exert bactericidal and bacteriostatic effects. This explained why there were activities on bacteria by the *Erythrina abyssinica* plant extracts.

Understanding the spectrum of antibacterial activity indicated that bioactive substances had broad spectrum or narrow spectrum of activity. The degree of susceptibility (diameter of inhibition zone) showed that gram positive bacteria were more susceptible compared to gram negative bacteria. There was urgent need to undertake phytochemical analysis of *Erythrina abyssinica* extracts to determine the chemical bioactive substances present in the extracts.

Minimum inhibitory concentration (MICs)

The root and stem barks of *Erythrina abyssinica* had activity against *Staphylococcus aureus* in all districts except stem bark for Rakai. The root barks, stem barks and leaves of Mbarara, Bushenyi respectively were effective against *Pseudomonas aeruginosa* demonstrated in Table 3. This demonstrated that the leaves extracts do not have antimicrobial activities.

Traditionally, the results from in vitro antimicrobial tests are written as qualitative or quantitative. Qualitative results are reported as susceptible, intermediate or resistant,

whereas quantitative results are reported as minimum inhibitory concentration (MIC) in µg/ml or g/ml or mg/l (Walker, 2006).

Walker,(2006), indicated that in-vitro antimicrobial susceptibility tests were predictive of in vivo therapeutic efficacy. However the ability of an in-vitro test to predict the clinical effectiveness of an antimicrobial agent is dependent on that test being performed correctly.

In vitro tests involve the continuous exposure of a relatively small concentration of bacteria to a constant level of antimicrobial agent under standardized testing conditions (Walker, 2006).

The selection of an appropriate dose can be driven by the result of quantitative susceptibility tests (Ambrose, 2005). Studies in human medicine have demonstrated the clinical value of in vitro susceptibility tests.

The interpretation of susceptibility testing depended on the relationship between in vitro susceptibility and factors involved in relation to tissue drug concentration (which depended on factors such as dose and pharmacokinetic and pharmaco-dynamic properties of the drug or drug class.

2.7.2 Crude extracts of leaves and fruits of *Capsicum annum*

2.7.2.1 Yields of the plants extracts

This study found out that the yields of the leaves extracts of *Capsicum annum* were better than the fruits as seen in Table 1 and Figure 2. There is an average 58% yield for the leaves compared to 10-11% yields for the fruits in all the districts of Ntungamo, Mbarara, Bushenyi and Rakai. The information gained on the percent yields of crude extracts are used for standardizing dosage rates of fine powder preparations of the plant materials. For example, the amount of crude extract contained in a known weight of fine powder of plant materials can then be calculated. This agrees with findings by (Ejobi F and Olila D 2004; Olila et al., 2007).

2.7.2.2 Antibacterial activities of the plant extracts

The antimicrobial activity of crude extracts of fruits of *Capsicum annum* was positive for *Salmonella species* and *Pseudomonas aeuroginosa* in Mbarara and Rakai respectively as detailed in table 2. There were no microbial activity by leaves of *Capsicum annum* in all the districts of Ntungamo, Mbarara, Bushenyi and Rakai. The fruit extracts did not have any activity on *Staphylococcus aureus* and *E.coli*.

The attributing factor for anti bacterial activity of the crude fruit extracts could be due to presence of bioactive constituents' in the extracts. This agrees with findings by Masola *et al.*, 2009 and (Ogundare et al., 2006).

A study by Masola *et al.*,2009 in Mpwapwa, in the semi arid central zone of Tanzania found out that bioactive constituents of terpenoids, tannins, phlobotannins, saponins and cardiac glycosides were found to be present in the stem barks of the plant *Adansonia digitata* (Bombacaceae) (African baobab).

Further still, studies by Ikigai et al., 1993 indicate that purified tannins, saponins and terpenoids have anti microbial activity. These bioactive compounds were reported to be effective against gram positive and gram negative bacteria. They exert bactericidal and bacteriostatic effects.

Understanding the spectrum of antibacterial activity will indicate if given bioactive substance had broad spectrum or narrow spectrum of activity. The degree of susceptibility (diameter of inhibition zone) will show that gram positive bacteria are more susceptible compared to gram negative bacteria. The need to undertake phytochemical analysis of fruit extracts of *Capsicum annum* to know the chemical bioactive substances present in these extracts.

2.7.2.3 Minimum inhibitory concentration (MICs)

The fruit extracts of *Capsicum annum* from Bushenyi and Rakai exhibited minimum inhibitory concentration against *Salmonella species* and *Pseudomonas aeruginosa* respectively as detailed in table 4. There was no minimum inhibitory concentration noted for the case of leaves of *Capsicum annum*.

Traditionally, qualitative and quantitative results from in vitro antimicrobial tests are reported. Qualitative results are reported as susceptible, intermediate or resistant, whereas quantitative results are reported as minimum inhibitory concentration (MIC) in µg/ml or g/ml or mg/l (Walker, 2006).

Walker,(2006), noted that in-vitro antimicrobial susceptibility tests are predictive of in vivo therapeutic efficacy. However the ability of an in-vitro test to predict the clinical effectiveness of an antimicrobial agent is dependent on that test being performed correctly.

| Plant Sample | Location | Plant part | Weight of sample (g) | Dry weight of concentrate (g) | % yield |
|-----------------|----------|------------|----------------------|-------------------------------|---------|
| <i>C. annum</i> | Mbarara | Leaves | 146.8 | 37.1 | 25.3 |
| | Bushenyi | Leaves | 62.4 | 53.9 | 86.4 |
| | Ntungamo | Leaves | 162.5 | 84.2 | 51.8 |
| | Rakai | Leaves | 253.0 | 174.0 | 68.8 |
| | Mbarara | Fruits | 85.3 | 8.8 | 10.3 |
| | Bushenyi | Fruits | 127.1 | 11.6 | 9.1 |
| | Ntungamo | Fruits | 61.3 | 4.9 | 8.0 |
| | Rakai | Fruits | 248.9 | 37.0 | 14.9 |

Table 4. Percentage yield extracts of the leaves and fruits of *Capsicum annum* from Mbarara, Bushenyi, Ntungamo and Rakai districts

In vitro tests cannot always predict the efficacy of an antibacterial agent in vivo (Walker, 2006). In vitro tests involve the continuous exposure of a relatively small concentration of bacteria to a constant level of antimicrobial agent under standardized testing conditions. The selection of an appropriate dose can be driven by the result of quantitative susceptibility tests (Ambrose, 2005). Despite these considerable differences, studies in human medicine have demonstrated the clinical value of in vitro susceptibility tests (Ambrose, 2005).

By generating full range MICs, a laboratory can give clinicians information that may allow them to individualize the therapeutic regimen, especially with regard to dose and dosing frequency. For example, if the MIC is low, the dose or frequency of dosing may be decreased. On the other hand, if the MIC is higher, but the organism is still considered susceptible and the drug has a wide pharmacotoxicity margin, a higher dose of the drug may be used.

Interpretation of susceptibility testing depends on knowing the relationship between in vitro susceptibility and factors involved in relation to tissue drug concentration (which depend on factors such as dose and pharmacokinetic and pharmacodynamic properties of the drug or drug class).

3. Conclusions

The root and stem barks of *E. abyssinica* have better yields than their leaves. The root and stem barks exhibit antimicrobial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The study had demonstrated that the leaves extracts generally did not have antimicrobial activities. The MICs of the root and stem bark extracts ranged from (3.5-31.3) mg/ml for *Staphylococcus aureus* and (410-1000) mg/ml for *Pseudomonas aeruginosa*. The study agreed that farmers were right in using root barks of *E. abyssinica* to treat various ailments in poultry diseases including other livestock diseases. There is need for further research to do phyto-chemical analysis to analyze the bioactive constituents of the extracts, undertake acute toxicity tests of the extracts.

It was clear that *Capsicum annum* have antibacterial activities against *Salmonella species* and *Pseudomonas aeruginosa*. The results reveal that the fruit extracts of *Capsicum annum* have activity against *Salmonella species*, *Pseudomonas aeruginosa* in Mbarara and Rakai districts respectively. No activity was noted against *E. coli* and *Staphylococcus aureus*. The leaves of *Capsicum annum* did not show any antibacterial activities. The MICs of the fruit extracts was 1000mg/ml. The study agreed that farmers may be right in using fruits of *Capsicum annum* to treat various ailments in poultry diseases. There is need for further research to do phytochemical analysis to analyze the bioactive constituents of the extracts, undertake acute and chronic toxicity tests for *Capsicum annum* extracts.

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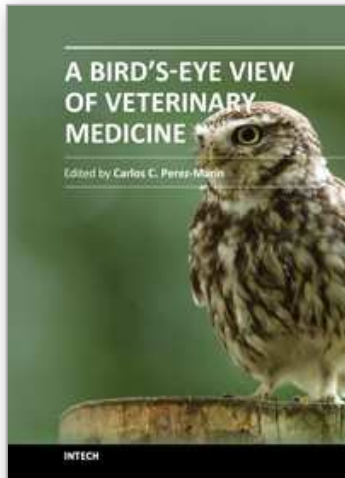
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