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Potential of Essential Oils in Filling Gaps and Removing Traps for Sustainable Poultry Production

Christabel Aghorowei[1], Amamieghaye Edum[1], Ruth Tariebi S. Ofongo[1]
[1]Niger Delta University, Department of Animal Science, Faculty of Agriculture, Niger Delta University, Wilberforce Island, P.M.B. 071 Yenagoa, Bayelsa State Nigeria.

Introduction

Phytobiotics or plant extracts are promising alternatives to antibiotic growth promoters (AGPs) in the poultry industry. This can be attributed to the high content of pharmacologically active compounds present in them. Plant extracts and essential oils utilization has yielded varied and interesting responses in poultry and pig (Zhai, *et al.*, 2018). These responses are varied in terms of growth performance, nutrient bioavailability, impact on gut health and gastrointestinal tract (GIT) responses, effectiveness against specific bacteria in named animal species as well as immune response. Previous, *in-vitro* studies with *Ocimum gratissimum* (*lyn*) and ginger extract indicated great potential against certain zoonotic bacteria (*Salmonella sp, Escherichia coli – E. Coli* – Ohimain *et al.,* 2015; Zige and Ofongo 2019 and of recent *Clostridia* – Akpan and Ofongo - Abule, 2019.
A shortage of documented physiological and microbiological effects in vivo (Applegate *et al.*, 2010) may be a factor limiting the addition of phytogenics as feed additives in certain instances. Several studies have demonstrated that essential oils have several properties, such as antimicrobial, anti-oxidative and anti-inflammatory effects, feed palatability enhancement and improvement in gut growth and health. There is still a need of further investigations to elucidate the mechanisms underlying their functions (Omonijo *et al* 2018). According to Vinus *et al* (2018), most of them elicit antibacterial, coccidiostatic, anti-helminthic, anti-viral, anti-inflammatory and in particular exhibit antioxidant properties. As a new class of additives to animal and poultry feeds, a wide variety of medicinal plants exist in nature and will need to be studied extensively to enable their usage as natural feed additives for poultry.
This study was carried out to determine the in-vitro antibacterial activity of the essential oil of *O. gratissimum* (lyn) against *Salmonella enteritidis*, *Salmonella typhymurium* and *Clostridia perfringens* isolated from poultry litter.

Material and Methods

Preparation of plant leaves and extraction of essential oil
Fresh *O. gratissimum* (lyn) leafs were harvested first thing in the morning. The leaves were separated from the stalk, placed in zip lock bags and taken to the laboratory for extraction of the essential oil by hydro – steam distillation method.
Since essential oils are volatile (quickly evaporating) aromatic fluids, hydro – steam distillation method with a Clevenger Apparatus. This technique uses heat to separate the aromatic oil from an organic source. This technique conducts the distillation process by boiling, condensing and decantation to separate the oil from the organic source. Hydro-distillation works because the water and the oil are immiscible. Hence, they boil independent of each other.

The Fresh leaf samples were washed in distilled water lightly, weighed and packed in the round-bottom flask then distilled water was added at an appropriate ratio depending on the nature/density of the sample). The set-up was put together and heat applied for 2 to 4 hours. The essential oil was collected in an air-tight amber bottle and stored in the refrigerator until usage.

In – vitro antibacterial activity

The essential oil was standardized to 80 %, 40 % and 20 % respectively with the aid of pharmaceutical grade laboratory soap. To 8 ml of *O. gratissimum* essential oil, 2ml of 2% laboratory soap solution which served as diluent was added. This gave 80 % essential oil. The 40 % and 20 % standard was prepared appropriately and they all served as working samples of the essential oil.

The assay organisms; *Salmonella enteritidis, Salmonella typhimurium*, and *Clostridium perfringen* were primarily isolated on various diagnostic media accordingly then sub cultured further to obtain pure culture. *Clostridium perfringens* was cultured on Reinforced Clostridia Agar while *Salmonellae* were sub cultured on *Salmonella shigella* agar onto Mueller Hinton Agar to remove the effects of indicators and suppressive chemical agents in primary isolation media. *Salmonellae* were then sub cultured into sterile nutrient broth for optical density calibration. Reinforced clostridia broth was prepared from the clostridia agar by allowing the agar gelling agent to settle and then decanted. Incubation periods were 24 hours for all the bacteria at 37°C. The disc diffusion method was used to determine the zone of inhibition (ZOI — in mm) of *O. gratissimum* essential oil against the 3 bacteria. One hundred and fifty micro litre (150 µl) of the various working concentrations was dispensed into the agar wells and allowed to stand for four hours before incubation. All the Petri dishes were incubated lid-up position in order to avoid spillage. After four hours on the Laboratory bench during which the samples and standard concentration were allowed to diffuse, the plates for antibacterial studies were incubated at 37^0C and observed after 24 hours then observed for zones of inhibition as a result of growth of specific bacteria sampled. Several readings were taken with zone reader and average zone values were determined and recorded. Clostridium plates were incubated anaerobically. Several readings were also taken with zone reader and average zone values were also determined and recorded.

Results and Discussion

Results obtained from the in – vitro antibacterial activity showed that o. gratissimum essential oil has antibacterial activity against *S. enteritidis*, *S. typhymurium* and *Clostridia perfringens* respectively (fig 1). The least zone of inhibition (ZOI) of 23mm was recorded at 20 % concentration of the essential oil against *S. enteritidis.* A value of 25mm and 28mm which was also the least was recorded at 40% and 80% concentration of the essential oil against *S. enteritidis*. The values recorded against *S. typhymurium* at 80% concentration was 40mm. values recorded at 40 % and 20 % was 35mm and 25mm respectively. These values were higher than that recorded for *S. enteritidis*. The essential oil cleared *C. perfringens* at all levels of treatment. The ZOI recorded against *C. perfringens* at all levels of treatment was > 40.00mm. Earlier *in – vitro study* with *O.* gratissimum (lyn) oil extracted from the aerial parts (leaves and flowers) freshly harvested using petroleum ether as solvent was reported to elicit antibacterial effect against *Salmonella sp* and *Clostridia perfringens* isolated from different sections of the GIT of six weeks old broiler chickens (Akpan and Ofongo – Abule 2019). Results obtained from that study showed inhibition of *Salmonella Spp* growth by 14.5 ± 1.92mm in the duodenum, jejunum and caecum. A value of 14.5 ± 2.06mm was recorded against *Clostridia* in the jejunum which was the highest recorded against *Clostridia*. There was no significant difference ($p>0.05$) regarding which bacteria the oil was most effective against in that study. In a previous study, (Ohimain *et*

al., 2015) a ZOI of 11.8mm was reported against *Salmonella* isolated from the ileum of broilers when *O. gratissimum* extract was used. Evidently, *O. gratissimum* essential oil resulted in higher ZOI against the three specific bacteria tested as supposed to its petroleum ether extract. The essential oil, at all levels of concentration cleared the growth of *Clostridia perfringens*.

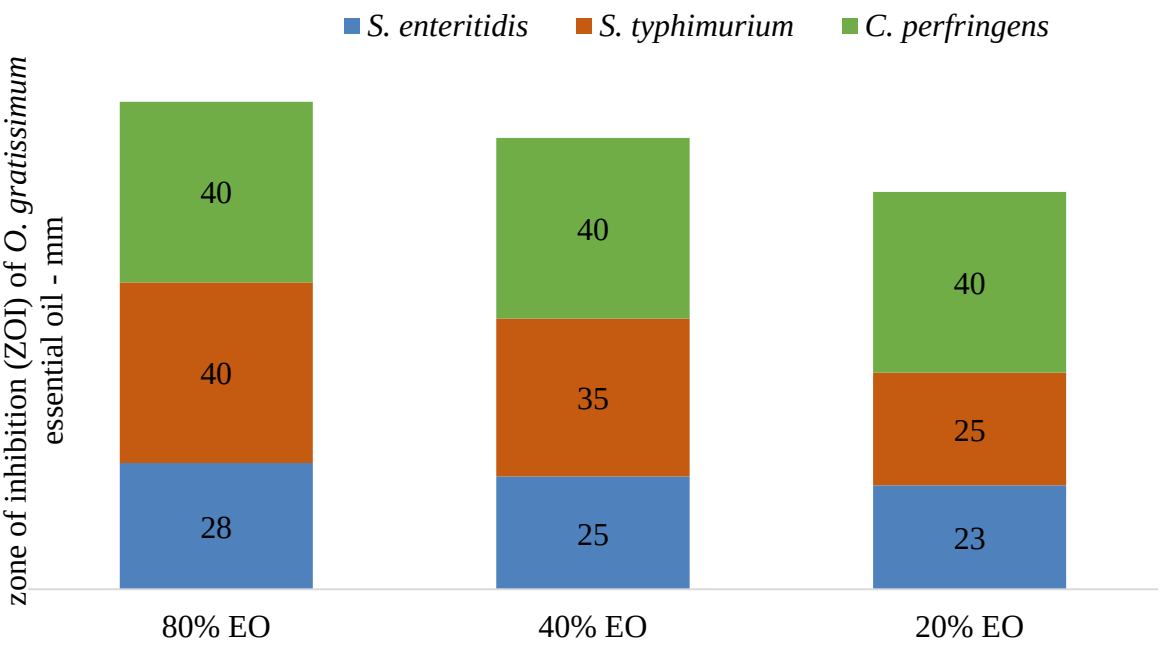

fig 1:Antibacterial activity of *O. gratissimum* essential oil (EO)

Earlier reports by Bankole *et al*. (2012) reported a ZOI of 7 – 15mm using a concentration range of 25 – 75% *Ocimum* water extract. Other authors have also reported lower ZOI of 10mm against *Salmonella* as reported by (Oladapo *et al.*, 2010) with concentrations of 20 – 30mg/ml water extract of *Ocimum.* Other authors have reported values higher than 14mm and 7mm at varied concentrations (Adebolu and Oladimeji, 2005; Olamide and Agu, 2013; Matasyoh *et al*., 2013). Ewing (2008), stated that the greater the ZOI, the stronger the antimicrobial effect. It is obvious from the results of the current study that, *O. gratissimum* essential oil had a stronger antibacterial effect against *S. enteritidis*, *S. typhymurium* and *C. perfringens* than the other extracts previously tested against *Salmonella* and *Clostridia*.

Conclusions and Outlook

From the results obtained in this study, it can be concluded that *O. gratissimum* essential oil has potential filling gaps and removing traps for sustainable poultry production. In the light of the results obtained from this study, in vivo studies to determine the efficacy of the essential with poultry can be carried out.

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