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Potential of phytogenics in filling gaps and removing traps for sustainable livestock production without antibiotics

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Introduction

Sustainable livestock production without antibiotics makes issues of pathogenic bacteria infections in farmed animals of utmost concern in situations of disease outbreaks that result in high mortality which subsequently affects profitability of production to farmers. According to reports, the long term and extensive use of antibiotics for veterinary purpose may eventually result in selection for the survival of resistant bacterial strain (Aarestrup 1999; Munk *et al*., 2018). Genes encoding for this resistance can also be transferred to other formerly susceptible bacteria, thereby posing a threat to both animal and human health (Munk *et al*., 2018). Ban on the use of in-feed antibiotics for farmed animals is a welcomed development with regards to antibiotic drug resistance in humans the consumers of farmed animals and their products. In the current era of antibiotic free animal production, medicinal plants and spices are being highlighted to fill gaps and remove traps for sustainable livestock resource development. Secondary plant metabolites such as tannins, saponins, flavonoids, anthraquinones and other compounds with antimicrobial, antibacterial, antifungal, antiviral and immune response inducing potential can successfully fill gaps in sustainable livestock resource development without antibiotic growth promoters. This will ultimately benefit both producers and consumers of animal products. Secondary metabolites such as tannins, polyphenols, flavonoids of plant origin with potential antimicrobial, anti-parasitic, anti-oxidant and anti-inflammatory (Wang *et al* 2008; Viveros *et al*., 2011 and Tosi *et al*., 2013; Brodowska, 2017) properties can be exploited in the livestock industry for sustainable production without antibiotics.

In order to better understand these potentials of phytogenics, qualitative and quantitative analysis of the methanolic extract of *O. gratissimum* (lyn) was carried out using standard methods.

Material and Methods

Sample preparation

Freshly harvested *Ocimum gratissimum* leafs were separated from the stalk and packed in zip lock bags and sent to the laboratory for analysis. The plant materials were washed lightly with distilled water and oven-dried at a temperature of 40°C for 72 hours until constant weight was observed. The dried leaves were then ground with an electric milling machine to powder.

Extraction

The *O. gratissimum* leaf powder was extracted with 80 % methanol (Sigma-Aldrich Analytical grade) using cold maceration method. The mixture of *O. gratissimum* leaf powder and 80 % methanol was vigorously stirred intermittently, then allowed to stand for 72 hours after which it was filtered through a Whatman number 1 filter paper-lined funnel into a conical flask. The

solvent from the filtrate was recovered using a rotary evaporator under vacuum at 40°C. The extract was further concentrated and dried using a water bath at 40°C for 48 hours.
The semi-solid crude extract was kept in a desiccator for 24 hours until constant weight was observed.
The extract obtained was kept in McCartney bottle and stored in refrigerator until required for use. The extract was analyzed for alkaloids, tannins, glycosides, steroids, flavonoids, saponins, anthraquinone, etc. using standard procedures.

Qualitative analysis

Qualitative analysis for specific phytochemical was carried out as follows: terpenoids (Liebermann – Burchard test); phenolic compound (Lead acetate test); Tannins (Ferric chloride test); flavonoids (Shinoda's test) and reducing sugars (Fehling's test).

Quantitative analysis

Analysis of the extract was carried out to determine the quantity of each phytonutrient found in the extract and the values reported on per gram basis.

Estimation of Phenolic Compounds content

The total phenolic content of the extract was estimated using the Folin Ciocalteu reagent as described by (Singleton and Rossi 1965). A calibration curve was plotted by mixing 1 ml aliquots of 50, 100, 150, 200, 250, 300, 350, 400 and 450 µg/ml Gallic acid solutions with 5.0 ml of Folin Ciocalteu reagent (diluted tenfold) and 4.0 ml of sodium carbonate solution (75 g/l). The absorbance was measured after 30 min at 765 nm. For the extracts (1 g/100 ml), 1 ml was mixed separately with the same reagents, as performed for constructing the calibration curve. After 1 h, the absorbance was measured to determine the total phenolic contents in the extract using the formula:

$T = (C \times V)/M$

Where, T = Total phenolic contents in milligrams of Gallic acid equivalent per gram of the extract C = Concentration of Gallic acid in mg/ml obtained from the calibration curve,

V = Total volume of extract used in assay,

M = Total weight of dry extract (in gram) used in the assay.

The total phenolic content of the extract estimated using the Folin Ciocalteu reagent method was reported as Gallic acid equivalent /g of the extract.

Total flavonoid determination

The method is based on the formation of flavonoids - aluminum complex which has an absorptivity maximum at 415 nm. A quantity (100 µl of the sample extract in methanol (10 mg/ml) was mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid. This mixture was then diluted with methanol to 5 ml. The absorption at 415 nm was read after 40 minutes of incubation. Blank samples were prepared from 100 ml of sample extracts and a drop of acetic acid, then diluted to 5ml with methanol. The absorption of standard Rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in triplicates.

Total flavonoid content was expressed as Rutin equivalents (mg Rutin Equivalents /g extract).

All analysis was carried out in triplicates.

Results and Discussion

The results obtained from the qualitative and quantitative analysis of secondary metabolites present in the methanolic extract of *O. gratissimum* is presented in Table 1.

Qualitative analysis revealed the presence of terpenoids, phenolic compounds and tannins which were (+++) heavily detected in *O. gratissimum* as indicated in the table below. Flavonoids was (++) detected in the extract also. Quantitative analysis of the extract indicated total phenolic concentration as Gallic acid equivalent to be 37.45 mg while flavonoid concentration as Rutin equivalent was 35.17 mg respectively.

Table 1: Qualitative and quantitative concentration of secondary metabolites in *O. gratissimum* methanolic extract.

Secondary metabolite	Level (concentration) /mg of extract
Terpenoids	+++ (heavily detected)
Phenolic compounds	+++
Tannins	+++
Flavonoids	++ (detected)
Total phenolic	37.45 mg Gallic acid equivalent
Flavonoid	35.17 mg Rutin equivalent

The phytochemical constituents in medicinal plants have been reported to play significant role or involved in the positive response obtained from studies utilizing medicinal plants as phytobiotics or natural growth promoters.

Tannins

Tannins are widely distributed in the plant kingdom and are notably abundant in nutritionally important forages, shrubs, cereals and medicinal herbs. New leaves and flowers of plants (vulnerable plant parts) are generally abundant in tannins. (Van Soest, 1982; Terrill *et al*., 1992; Frutos *et al*., 2004). They elicit, antimicrobial, anti-parasitic, antioxidant, anti-inflammatory and antiviral properties. Initially considered as anti-nutritional factors (Redondo *et al*., 2014) in monogastric diets (pig and poultry), recent reports indicate that low concentrations of several tannin sources improved health status, nutrition and animal performance in monogastric farm animals (Biagia *et al*., 2010; Starcevic *et al*., 2015). The result of this study further establishes *O. gratissimum* as a potential natural growth promoter for monogastric animals that can impact positively on gut health and growth performance. An earlier report stated the positive effects of tannins on promoting the health status of intestinal ecosystem through their anti-microbial, anti-oxidant and anti-inflammatory activities (Tosi *et al*., 2013).

Flavonoids

Flavonoids are characterized by antioxidant, pharmacological, anti-inflammatory, anti-allergic, antiviral, anti-carcinogenic, as well as therapeutic and cytotoxic properties (Brodowska 2017). Flavonoids have attracted a great deal of attention due to their potential health benefits. Supplementing flavonoids in poultry diets has shown its potential to progress the nutritional, sensorial and microbiological quality of poultry meat and eggs (Kamboh *et al* 2019). Flavonoids are normally absorbed in the ileum where pH is between 5 – 6.8. Aqueous extract of *O. gratissimum* was reported to significantly ($p< 0.05$) reduce pH in the ileum (6.36±0.03) and caecum (6.46±0.01) of broiler birds (Anugom and Ofongo 2019). Adequate absorption of flavonoids would imply its benefit expressed in various aspect of the birds' physiology and products obtained from birds that have consumed such flavonoids. Recent studies on flavonoids focused on benefits of flavonoids for inhibition of lipid oxidation and microbial growth, check any pH-dependent deterioration and improve the colour stability of meat and related products (Kamboh *et al* 2019). These studies are further pointers to the possibility of *O. gratissimum* as a source of flavonoids with prospective for use as suitable feed additive. Potential anti-oxidative and anti-inflammatory effects of polyphenols have been less investigated in farm animals so far (Gessner *et al* 2017). The occurrence and the consequences of oxidative stress and inflammation on animal health and performance can be alleviated with the use of flavonoids of plant origin. Thus further emphasizing the need to study the bioavailability and metabolism of polyphenols from *O. gratissimum* in livestock nutrition. The mode of action of plant extracts in poultry nutrition has not been fully understood or well elucidated. But modulation of gut health through their antibacterial activity (Ofongo and Ohimain, 2019) and immune stimulation are potential means of enhancing gut health. Any positive effect they have on gut health will ultimately lead to enhanced bird performance which is desirable to poultry farmers. The use of various plant

flavonoids as a substitute for synthetic feed additives in the poultry feed industry to satisfy consumer demands in terms of quality and safety of animal products cannot be over emphasized.

Conclusions and Outlook

From the results obtained, it is obvious that *Ocimum gratissimum* has secondary metabolites with potential to fill gaps in sustainable livestock resource development in the absence of antibiotics. Appropriate experimental trials targeting the use of *O. gratissimum* extract as alternative to antibiotics commonly used in livestock production will establish its potential in filling gaps for sustainable livestock production without antibiotics.

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