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Antimicrobial activity of *Ocimum gratissimum* Extract on *Suya* (an intermediate moisture meat) in Nigeria

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Abtract

Extract of Ocimum gratissimum leaves was used on Suya meat (an intermediate moisture meat) harvested at different hours of soaking period. O. gratissimum leaves were collected from Oyo state, South West region of Nigeria, rinsed in distilled water and squeezed to extract the fluid. The meat used was semimembranous muscle from beef carcass which was trimmed of all visible fat and connective tissues. The meat cut was sliced to sheets of 0.18cm-0.35cm thick and lengths of between 5.0cm-7.1cm. The study comprised of five treatments of 10 replicates each. Treatment A (TA) served as the control- (Suya without Ocimum Gratissimum Extract-OGE), while (TB),(TC),(TD) and (TE) were soaked in OGE for ½ hr, 1hr, 1½ hrs and 2 hrs respectively, before coating with Suya ingredients. A total of 50 sticks of Suya with an average weight of 38.10 - 59.30grams of sliced meat per stick were prepared for each treatment sample. The meats on sticks were properly coated with Suya ingredient.

The morphological and biochemical characterization of aerobic bacteria, coliform and lactic acid isolates from the five treatments was carried out. At Day 0: From samples of the five treatments were isolated, five (5) Aerobic species namely: Pseudomonas sp, Bacillus sp, Micrococcus sp and Flavobacterium sp. Three (3) Coliforms sp were also isolated namely: Proteus sp, Aeromonas sp and Enterobacter sp. Four (4) Lactic acid bacteria were also isolated namely: Pediococcus sp Streptococcus sp, Lactobacillus sp and Enterococcus feacalis. Suya meat soaked in OGE at different harvesting hours ½ hr, 1hr, 1½hrs and 2hrs, on the days ranged between 0.01 x10^5 to 0.07 x 10^5; 1.0 x 10^5 to 0.04 x 10^5; 0.1 x 10^5 to 3.0 x 10^5 and 0.01 x 10^5 to 0.2 x 10^5 respectively however, the microbial counts were relatively low at third and fifth days which might be as a result of the active chemotypes in OGE. Coliform counts for Day 7 for TA and TB were exceptionally high.

Introduction

O. gratissimum L. is an aromatic medicinal plant belonging to Lamiaceae family. It is an important herbal medicinal plant in Nigeria communities and also in the sub-Saharan Africa. The leaves are rubbed between the palms and sniffed as a treatment for blocked nostrils Kokwaro, (1993). They are also used for abdominal pains, sore eyes, and ear infections, for coughs, barrenness, fever, convulsions, tooth gargle, regulation of menstruation and as a cure for prolapse of the rectum (Watt and Breyer-Brandwijk, 1962; Harjula, 1980; FAO,1986; Kokwaro, 1993).

Several species and varieties of plants of the genus *Ocimum* have been reported to yield oil of diverse nature. *O. gratissimum* commonly known as basilica oils. Craveiro *et al.* (1981) and Janine de Aquino Lemos *et al.* (2005) reported some chemical compounds and active ingredients found in these plants such as eugenol, linalol, methyl cinnamate, camphor and thymol. Various species of *Ocimum* have been reported for their numerous medical uses Mshana *et al.*, (2000).

It is in line with this preservative ability of the plant that this work tried to explore the usefulness of *Ocimum gratissimum extract (OGE)* plant in *Suya* processing. *Suya* is an intermediate moisture meat products of West Africa that is easy to prepare and highly relished. There are three types of *Suya* namely: Tsire, Kilishi and Balangu of the three types Tsire that is boneless meat pieces that are staked on slender wooden sticks and cooked by roasting using a glowing fire, is certainly the most popular with consumers. (Igene and Mohammed, 1983).

Materials And Methods

Plant material: Fresh leaves of *Ocimum gratissimum* were collected from Abadina area of the University of Ibadan in the month of October, 2008. The plant was identified at the herbarium unit of the Botany Department in the University of Ibadan.

Extract Preparation: The fresh leaves collected were weighed (60kg) rinsed with distilled water, in order to access the extract; after slightly fine blending, the extract was squeezed in muslin cheese cloth. A brownish green juice of volume 1200ml was obtained and kept in air-tight bottles in refrigerator until ready to use the same day.

Experimental Design: In a completely randomized design, the experiment was conducted with five treatments with ten replicates each. A total of 50 samples were randomly allocated into the 5 treatments. Samples were obtained from these and used for the chemical and microbial analysis.

Meat Preparation

Raw fresh meat was collected from the Department of Animal Science slaughter slab in the University of Ibadan. The age of the animals was within the range of 3 – 4 years.

The cut of beef used in this experiment was taken from a portion of the semi membranous muscle from

singed beef carcasses. The meat was trimmed of all visible bones, fat and connective tissues. It was chucked cut into 12cm long and 6cm wide. The chucks were sliced into thin sheets of between 0.18cm and 0.35cm thickness in the same direction of the muscle fibre using a long knife with a very sharp blade.

Ingredient Preparation

Spices and other ingredients were obtained from Bodija market in Ibadan, Oyo State. These ingredients comprised of locally available spices and condiments. These ingredients were mixed together in this specific proportion to include groundnut cake (powdered) 52%, salt 8.5%, dried pepper 10%, curry 5%, magi 7.5%, groundnut oil 2% and ginger 10%. The *Suya* sticks were obtained from Sabo area of Ibadan, Oyo State.

Preparation of *Suya*

Labelled measured staked meats were spread on a flat tray for easy identification. A total of 50 sticks of meat were made for all treatments these comprised of Treatment A (control)-without *Ocimum* extract (*OGE*), Treatment B -meat soaked in *OGE* for ½ hr,Treatment C - meat soaked in *OGE* for 1hr, Treatment D -meat soaked in *OGE* for 1½ hrs and Treatment E -meat soaked in *OGE* for 2hrs. After soaking, all the staked meat were properly coated with *Suya* ingredients. The labelled meat sticks were then arranged round a glowing, smokeless fire made from charcoal. The distance of the sticks of meat from the fire was 25.96 ± 2.31cm. The meats on sticks were allowed to stay around the fire for 25 minutes with regular turning; intermittently groundnut oil was sprinkled on the meat while roasting continued.

The weight of each *Suya* stick was determined after roasting and this was used in calculating the percentage cooking loss and the product yield and samples from each of this treatment was taken for microbiological analysis.

Microbial Analysis

Preparation of Media

Four different culture media were used to carryout the bacteriological and mycological analysis. The Minimum Inhibitory Concentration (MIC) was determined as well as the Bactericidal / Bacteriostatic effects of the extract, and to also determine what microbes might be present in this product. Nutrient agar (NA) was used for general microbial analysis, MacConkey agar (MA) for coliform bacteria, (PDA) for moulds and DeMann Rogosa and Sharpe (MRS) for lactic acid bacteria.

Preparation of Different Agar Media:

Nutrient Agar

28g of nutrient agar was suspended in 100ml distilled water using a water bath at 100°C.

MacConkey Agar

52g of weighed medium was dissolved in 100mls distilled water in a conical flask dipped in a water bath.

Potatoes Dextrose Agar

39g of PDA was homogenized in 1 litre of distilled water using a water bath at 100°C. **Stains Used**

These included gram staining (crystal violet, logous, iodine, safranin, ethanol), lactophenol cotton blue.

Isolation Technique – Serial Dilution

Isolation was made from each *Suya* samples using the serial dilution methods of Meynelle and Meynelle (1970). One gram of the sample was pounded and mixed thoroughly in 9ml sterilized distilled water in McCartney bottle or test tube.

Isolation of Organisms on Nutrient Agar and Potatoes Dextrose Agar

This was done using the pour plate method. The plates containing the nutrient agar were allowed to stay overnight while that of potatoes dextrose agar was incubated for 3 days. Bacterial usually will grow on nutrient agar while fungi will grow on potatoes dextrose agar.

Morphological Studies

Colonies, which developed after incubation were examined for structural features such as elevation, size, surface form, degree of growth, opacity, edge, consistency, and pigmentation. Pure cultures of the micro organisms were obtained by repealed streaking on nutrient agar plates for bacteria and fungi isolates. Cellular characteristics of the pure culture of each isolated micro organism were examined under the microscope using the oil immersion objective after gram staining.

Results And Discussion

The morphological and biochemical characterization of bacteria (Aerobes), coliform and lactic acid isolates from the five treatments was carried out. TA - without *OGE* (Control) and (TB)-*Suya* soaked in *OGE* for ½ hr , (TC) – *Suya* soaked in *OGE* for 1 hr, (TD) – *suya* soaked in *OGE* for 1½ hrs and (TE)–*Suya* soaked in *OGE* for 2 hrs.

At Day 0 of the examination: Five (5) Aerobic species were isolated from samples from the five treatments, namely: *Pseudomonas spp* (*Putida* and *cepacia), Bacillus spp* (*Subtilis* and *licheniformis), Micrococcus Spp (Sapophyticus* and *Lepidemidis)* and *Flavobacterium spp (Aquatile).*

Three (3) Coliforms species were also isolated from the five treatments namely: *Proteus spp (Murabilis* and *Vulgaris*), *Aeromonas spp (hydrophila*) and *Enterobacter spp (aerogenes).*

Four (4) Lactic acid bacteria were isolated from the five treatments namely: *Pediococcus spp (acidilactis), Streptococcus Sp (feacalis), Lactobacillus spp (brevis, Plantarum, Casei, fermentium* and *acidiophilus*) and *Enterococcus feacalis.*

Despite the fact that all these microbes were detected in the *Suya* meat at first day of preparation (i.e Day 0), the effect of the basil (*OGE*) on the meat was very pronounced judging from the total bacteria count for each day observed. TA (control-without *OGE),* showed the highest number of microbes, starting from Day 0 to 7.

Suya meat soaked in *OGE* at different harvesting hours ½ hr, 1hr, 1½hrs and 2hrs, on the days ranged between 0.01 x10^5 to 0.07 x 10^5; 1.0 x 10^5 to 0.04 x 10^5; 0.1 x 10^5 to 3.0 x 10^5 and 0.01 x 10^5 to 0.2 x 10^5 respectively however, the microbial counts were relatively low at third and fifth days. Coliform counts for Day 7 for TA and TB were exceptionally high.

The low counts of the microbes must be as a result of the active chemotypes of *O. gratissimum* which was identified as eugenol as reported previously by (Nakamura *et al.*, 1999; Janine de Aquino Lemos *et al.*, 2005). The compound (eugenol) has been demonstrated to have both antibacterial (Nakamura *et al.*, 1999; Adebolu and Oladimeji, 2005) and antifungal (Janine de Aquino Lemos *et al.*, 2005) activities. The *Ocimum* oil is dominated by eugenol, which accounted for 68.81% of oil and methyl eugenol (13.21%). Minor components include cisocimene (7.47%), germacrene-D (4.25%), transcaryophyllene (1.69 %) and pinene (1.10%).

In another research conducted by (Celso *et al.*, 1999) he reported the inhibition zones of *OGE* determined for six strains of Gram-positive or Gram-negative bacteria using the diffusion technique on solid media. *Proteus, Klebsiella, Escherichia, Salmonella, Staphylococcus* and *Shigella* showed inhibition zones ranging from 13 to 25 mm. *P. aeruginosa* was considered resistant since no inhibition zone was observed. From this report the presence of the phenol active ingredient must have also contributed to the keeping down of the the microbial counts on the third and fifth days.

However, in another study conducted by Lexa *et al.*, (2006) the essential oil was evaluated for antimicrobial activity against pathogenic strains of Gram positive (*S. aureus, Bacillus spp.*) and Gram negative (*E. coli, P. aeruginosae, S. typhi, K. pneumoniae, P. mirabilis*) bacteria and a pathogenic fungus *C. albicans*. It was found to be active against all the bacterial strains activities. The fungus, *C. albicans,* was highly susceptible to the essential oil. Other studies showed that the essential oils (EO) of four *Ocimum* species grown in Rwanda ,i.e. *O. canum, O. gratissimum, O. trichodon* and *O. urticifolium*, display antimicrobial activity (Janssen *et al.* 1989). It has been reported that the volatile oil of this plant contains mostly phenols, particularly thymol (Olivier 1960, Sainsbury and Sofowora 1971) and that these are probably responsible for its reported antimicrobial action.

Conclusion

Results obtained in the above study shows that *Ocimum gratissimum* has potentials of being used in the meat industry and it has to be fully explored for preservative purposes

References

Adebolu T.T. Oladimeji S.A. 2005. Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhea causing bacteria in Southwestern Nigeria. Afr. J. Biotechnol. 4(7): 682-684.

Celso V. N., Tania U.N., Erika B., Abrahão F. N. M., Díogenes A. G. C., Benedito P. D. 1999. Antibacterial Activity of Ocimum gratissimum L. Essential Oil

Craveiro A.A., Fernandes A.G., And Matos F.J.A and Alencor Oleosessenciais de plantas dc Imprensa Universitaria, Universic do Ceard, Fortaleza.

FAO 1986. Some Medicinal Forest Plants of Latin America.Forestry Paper 67

Harjula H 1980. Mirau and his practice: A st ethnomedicinal repertoire of a herbalist Tri-med. books London p. 2.

Igene, J.O and Mohammed, I.O 1983. (preferences and attitude to s indegeneous meat products .Annals c 169.

Janine de Aquino Lemos, Xisto S. P., Orion Fatima L. F, Jose Realino de Paula, P F., Lucia Kioko Hasimoto de Souza, de Aquino Lemos, Maria de R Rodriques Silva 2005. Antifungal ac from *Ocimum gratissimum* L. to *Cryptococcus neoformans*. Mem. Oswaldo Cruz.100 (1): 55-58.

Janssen A.M., Scheffer J.J., Ntezurubanza Baerheim Svendsen A. 1989. Antimicro acitivities of some *Ocimum* species grown Rwanda. *J Ethnopharmacol 26*: 57-63.

Kokwaro J.O. 1993. Medicinal plants of East Africa, Ea Africa Literature Bureau, Kampala, Nairob and Dar-es-Salaam. P. 106-115.

Lexa G. M., Josphat C. M., Francis N. W., Miriam G K., Anne W. Thairu M. and Titus K. M 2006. Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* .

Meynelle G.G and Meynelle E. 1970. Theory and practices in Bacterial. 2nd ed.CambridgeUni.Press. P.347.

Mshana N. R., Abbiw D.K., Addae-Mensah I., Adjanohoun E., Ahji M.R.A., Enow-Orock E.G., Gbile Z.O., Naomesi B.K., Odei M.A., Adenlami H., Oteng-Yeboah A.A., Sarppony K., Sofowora A., Tackie A.N. 2000. Traditional medicine and pharmacopoeia contribution to the revision of Ethnobotanical and Floristic Studies in Ghana, Scientific, Technical and Research Commission of the Organisation of African Unity.

Nakamura C.V, Nakamura T.V, Bando E, Melo A.F.N, Cortez D.A.G., Dias Filho B.P 1999. Antibacterial activity of *Ocimum gratissimum* L. essential oil. Mem. Inst. Oswaldo Cruz., 94: 675-678.

Oliver B. 1960. *Medicinal Plants in Nigeria*, Nigerian College of Arts, Science and Technology, Nigeria, 42 pp.

Rodriques S. 2005. Antifungal activity from *Ocimum gratissimum* L. towards *Cryptococcus neoformans*. Mem. Inst. Oswaldo Cruz.100 (1): 55-58.

Watt J.M., Breyer-Brandwijk M.G 1962. Medicinal and poisonous plants of Southern and Eastern Africa. E&S. Livingstone, Ltd. London.

Table 1: Total bacteria count of *Suya* prepared with *Ocimum gratissimum* extract.

Trt.A (No*OGE*)	Day 0	Day 3	Day 5	Day 7
Aerobes	0.2×10^5	2.22×10^5	2.2×10^5	0.2×10
Coli- forms	0.05×10^5	0.7×10^5	0.7×10^5	7.5×10^5
Lactic Acid Bacteria	0.03×10^5	0.5×10^5	0.5×10^5	1.1×10^5

Trt. B (½hr*OGE)*	Day 0	Day 3	Day 5	Day 7
Aerobes	0.02×10^5	0.1×10^5	$0..002 \times 10^5$	0.001×10^5
Coli- forms	0.03×10^5	0.02×10	0.02×10^5	6.0×10^5
Lactic Acid Bacteria	0.02×10^3	0.02×10^5	0.2×10^5	1.0×10^5

Trt.C (1hr. *OGE)*	Day 0	Day 3	Day 5	Day 7
Aerobes	0.02×10^5	0.002×10^5	2.2×10^5	0.2×10^5
Coli- forms	0.02×10^5	0.002×10^5	0.02×10^5	0.04×10^5
Lactic Acid Bacteria	0.01×10^5	0.04×10^5	$0.02.x\ 10^5$	1.0×10^5

Trt.D (1½hrs.*OGE*)	Day 0	Day 3	Day 5	Day 7
Aerobes	0.01×10^5	$0.02 .x\ 10^5$	0.2×10^5	0.1×10^5
Coli- forms	0.02×10^5	0.2×10^5	0.3×10^5	0.2×10^5
Lactic Acid Bacteria	0.002×10^5	0.02×10^5	02×10^5	3.0×10^5

Trt.E (2hrs.*OGE)*	Day 0	Day 3	Day 5	Day 7
Aerobes	0.01×10^5	001×10^5	0.1×10^5	0.1×10^5
Coli- forms	0.02×10^5	0.02×10^5	0.02×10^5	0.2×10
Lactic Acid Bacteria	0.002×10^5	0.01×10^3	0.02×10^5	0.2×10^5