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Microbiology and Pathology



Highlights

- Alternative of Antimicrobial Agent
- Effects of Antimicrobial Application
- Classification of CNS Tumors Criteria
- Annona Cinerea Dunal Grown in Nigeria

Discovering Thoughts, Inventing Future

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Inter-Observer Variation in the Grading of Meningiomas using the WHO Classification of CNS Tumors Criteria

By Hisham Alkhalidi

King Saud University

Abstract- *Background:* Grading of meningiomas using the World health organization (WHO) Classification of the Central Nervous System criteria currently has an essential role in classification, treatment, prognosis prediction, and research of these tumors.

Aims: This is a retrospective study that assessed the interobserver variation between Anatomical Pathologists in grading meningiomas using material obtained from ten resection specimens. The WHO grading system includes different methods, including the mitotic count, the tumor subtypes or the presence of three out of five certain morphological features. This paper focuses on the interobserver variability in the latter method.

Methods: Meningiomas that were originally graded based upon mitoses, brain invasion, or morphological subtype were excluded. Ten different Anatomical Pathologists, including two Neuropathologists, who were blinded to the original diagnosis and grade graded the tumors independently.

Keywords: *meningioma, radiation therapy, interobserver variation.*

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Inter-Observer Variation in the Grading of Meningiomas using the WHO Classification of CNS Tumors Criteria

Hisham Alkhalidi

Abstract- ***Background:*** Grading of meningiomas using the World health organization (WHO) Classification of the Central Nervous System criteria currently has an essential role in classification, treatment, prognosis prediction, and research of these tumors.

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Methods: Meningiomas that were originally graded based upon mitoses, brain invasion, or morphological subtype were excluded. Ten different Anatomical Pathologists, including two Neuropathologists, who were blinded to the original diagnosis and grade graded the tumors independently.

Results: There was "intermediate to good" interobserver agreement between the Pathologists using this method. The kappa score for interobserver agreement between the ten Anatomical Pathologists was 0.53, and between the two Neuropathologists was 0.55, with an overall agreement percentage of 70%.

Conclusions: More precise grading criteria and definitions can improve the interobserver agreement. Clinicians and researchers need to understand the difficulty in grading some meningiomas.

Keywords: meningioma, radiation therapy, interobserver variation.

I. Introduction

Meningiomas are relatively common dura-based tumors that constitute about 25-30% of primary brain tumors in Saudi Arabia (1,2). Grading of meningiomas has an essential role in patient management, including classification, treatment and prognosis prediction. Tumors with a higher grade have more chances of increased recurrence and mortality rates (3). The treatment of atypical and anaplastic meningioma is based on surgery and radiation therapy (4). The updated 2016 edition of the World Health Organization (WHO) Classification of Tumours of the Central Nervous system (5) provides the criteria used in the classification of meningiomas into three grades (from grade I to grade III). These criteria include the subtype of the tumor, brain invasion or mitotic counts, in addition to the presence of an overtly malignant high-grade morphology (i.e. sarcomatoid, carcinomatoid or melanoma). If three out of specific five criteria are present in a given meningioma, it should be graded as grade II. These five criteria are increased cellularity, small cells change with high N/C ration, large and prominent nucleoli, patternless or sheet-like growth and foci of spontaneous or geographic necrosis. These criteria do not clarify a quantitative definition. This paper explores the responses of ten pathologists who examined ten cases of meningiomas, where grading variability may occur due to different interpretations of these five criteria.

II. Method

In this retrospective study, forty-two meningioma cases were retrieved from the archives of the histopathology unit at King Khalid University Hospital, Riyadh, from 2017 to 2019. Excluded cases included any atypical or anaplastic meningiomas that showed brain invasion or were graded based on the increased mitotic count. Any meningioma that was graded as grade II or grade III based on the morphological subtype was also excluded. These subtypes are clear cell, chordoid, papillary and rhabdoid. Five grade I meningiomas were randomly selected, based on the original pathology report. Another five grade II meningiomas were randomly selected. The number of blocks for each case varied from one to fourteen, which can be attributed to the variation in the volume of tumor tissue submitted for histopathological assessment. All biopsies were processed and stained using routine hematoxylin and eosin stain. An experienced Neuropathologist reviewed each case and selected a representative slide. Ten experienced Anatomical Pathologists reviewed the selected slides and recorded the grade. The pathologists include two experienced Neuropathologists. The review was performed without knowledge of the previous clinical, radiological, or histopathological findings of the ten patients from whom those biopsies were obtained. A detailed educational

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sheet was used, expecting each pathologist to follow the WHO (2016) criteria for Meningioma grading. The results obtained from each pathologist were independently documented as meningioma (grade I), atypical meningioma (grade II) or anaplastic meningioma (grade III). A Neuropathologist recorded the morphological features of the ten cases. The results were tabulated and analyzed by the multiple-reader Cohen kappa statistical analysis method using a website based calculator (6) and a free-marginal multi-rater kappa. The aim was to assess the precision of agreement between the various observers (interobserver agreement).

III. Results

The results obtained by the ten participating pathologists and the two neuropathologists are summarized in Table 1. The kappa score for interobserver agreement between the ten anatomical pathologists was 0.53 (95 % CI for free-marginal kappa [0.32, 0.74]) with an overall agreement percent of 68.44%. The kappa score for interobserver agreement between the two neuropathologists was 0.55 (95 % CI for free-marginal kappa 0.1, and 1) with an overall agreement percent of 70%. Both kappa scores are in keeping with above chance "intermediate to good" agreement (7). The cases that showed 100% agreement were three cases. Two cases were WHO grade I. The third case was WHO grade II (Case 2, Figure 1). The latter was the only meningioma from the selected cases that showed focal necrosis. Another five cases were graded as either WHO grade I or WHO grade II by the reviewers. These cases were showing a variable degree of small cell change, lack of pattern, and cellularity (Figure 2).These cases did not show features of necrosis or prominent nucleoli. Two cases (Cases 8 and 9) were labelled as WHO grade II by the majority of the reviewers. However, in each one of them, two reviewers labelled them as WHO grade III (Figure 3). In their opinion, the reason for such designation was the focal presence of sarcomatoid morphology.

IV. Discussion

Meningiomas grading has an essential role in patients management and related research studies (8). The risk of recurrence further increases with WHO grade. In one study, patients with benign, atypical, and malignant meningiomas had a 10-year cumulative incidence of recurrence of 6%, 17%, and 30%, respectively. The 10-year relative survival of patients with WHO grade I, II, and III meningiomas were 97%, 90%, and 30%, respectively. These numbers demonstrate the significant increase in tumor-related mortality based upon the WHO grade (3). There is no clear recommendation about the use of radiation therapy in meningiomas (9). However, the higher grade the tumor, the more chances that the patient will receive adjuvant therapy (e.g., External Beam Radiotherapy). Surgical resection extent is the most important prognostic factor among malignant meningioma patients (3, 10). The extended safety margins are necessary to achieve a favorable local control for high-grade meningiomas (11). 15 to 80% improvement of the 5-year progression-free survival was reported when RT was added to surgical resection for malignant meningioma. Atypical meningiomas appear to be more frequently diagnosed under the WHO classification system updates (12). No consensus exists for "atypical" meningiomas treatment, and radiation therapy has mostly been reserved for recurrence and progression (13,14). Gross total resection and adjuvant radiation therapy appear to be highly associated with improved survival, independent of other factors, in patients with atypical meningiomas (15). Overall, the grading of meningiomas is essential and has a significant impact on both the clinical research studies and the treatment of these tumors.

Among the ten practicing pathologists and the two neuropathologists, this study's findings show that the inter-observer agreement on the grading of meningioma that is based upon the presence of the specified three out five features is "intermediate to good" above chance. Clinicians and researchers should be aware of this issue and the subjectivity element in the grading criteria. Three cases out of ten had a perfect agreement. Seven cases had discrepancies, while five cases were graded either as grade I or grade II, and two cases were essentially graded as grade II or grade III.

This paper does not incorporate grade II and grade III meningiomas that were classified based on relatively more objective criteria, including the brain invasion or the specific tumor subtype Morphology. Similar studies are limited in this field. In one study, the mitotic count is considered an objective method of grading, but variation in the grading using mitoses has been reported between pathologists based upon the number of fields examined (16). Another study (17) using previous WHO versions showed high concordance between the pathologists for brain invasion, ≥20 mitoses/10 high -powered field and spontaneous necrosis. The concordance was lowest for small cells, sheeting and ≥4 mitoses/10 HPF. For atypical meningioma, the criteria of diagnosis include the presence of the three out of five morphological features (3 out 5), as mentioned earlier. The case that displayed necrosis was the only one that had a perfect agreement as grade II. It appears that necrosis presence was a feature that prompted all the pathologists to look for more needed features to label the tumor as grade II during the case screening. However, the rest of the five features appears to be more problematic. The WHO criteria do not state the percentage of the tumor area, showing the features that are needed to apply criteria. Furthermore, the features

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are needed to apply criteria. Furthermore, the features do not have a quantitative definition. For example, regarding "increased cellularity", the criteria do not mention how many cells are needed per space unit to consider the tumor cellular. For "small cell change", the N:C ratio that should be present to consider a tumor cell as a small cell is not mentioned. "Prominent nucleoli" is left for the pathologist's judgments. In other body systems, the magnification power is used as a quantitative method for defining visible, prominent nucleoli (18).

For grade III tumors, one of the defining grading methods is the presence of sarcomatoid or carcinomatoid morphology. No statement of the volume of the tumor that should show this feature is clarified in the WHO criteria. Besides, these patterns may have a room for personal interpretation and opinions diversity. This explains the two cases where two pathologists labeled them as grade III, while the majority of the remaining pathologists graded them as grade II.

V. Conclusion

Atypical and anaplastic meningiomas can be challenging diseases, not only from a treatment perspective but also from a diagnosis perspective. As demonstrated, the current Meningioma grading system provided by the WHO book does not draw a sharp line between the different grade categories in a significant subset of meningiomas, and more precise criteria and definitions can help. This issue is particularly applicable to the following features: cellularity, lack of pattern, small cell change, prominent nucleoli and sarcomatoid or carcinomatoid morphology. A significant difference in interpretations may make it difficult to establish a definitive cut off that would translate accurately from one laboratory to another. Hence, clinicians and researchers should be aware of this concern. Understanding the grading criteria and the pathology report and communication with the pathologist are an essential element of meningiomas management. These tumors are signed out by general Anatomical Pathologists in many places. Hence, they should also be aware of the meningioma grading criteria and related-concerns. The pathology reports should include the basis of the grading and any difficulty that is associated with it. Besides, intradepartmental consultations, Pathologist's education and joint reporting by two experienced pathologists can help to maintain a high level of grading concordance. The biological signature of meningiomas is likely to play a significant role in the evolution of the grading system strategy. Hopefully, studies focusing on the immunohistochemical and genetic features of these complex tumors and relating these features to the treatment response and the prognosis will provide a more reproducible system with better concordance between pathologists and laboratories.

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Table 1: Grading responses of ten different meningiomas samples by ten Anatomical Pathologists (AP) including two Neuropathologists (NP)

Cases	NP	NP	AP	AP	AP	AP	AP	AP	AP	AP
1	1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2	2	2	2
3	1	2	1	1	2	2	1	2	1	1
4	1	1	1	1	1	1	1	1	1	1
5	2	2	2	2	1	1	2	2	1	2
6	1	1	1	1	1	1	2	2	1	1
7	1	1	1	1	1	1	2	2	1	2
8	2	2	2	2	2	2	2	3	2	3
9	3	2	2	2	1	2	2	3	2	2
10	1	2	1	1	1	1	2	2	1	1

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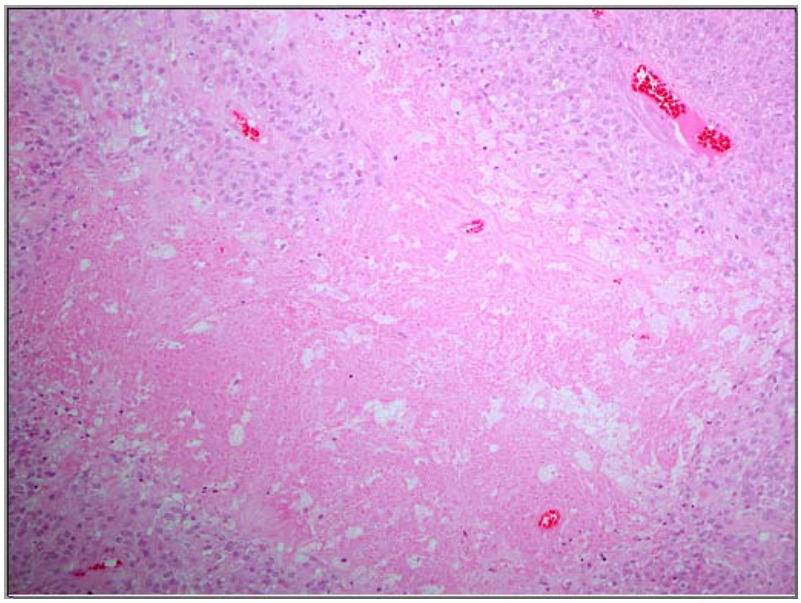


Figure 1: Necrosis in a grade II meningioma. The surrounding viable meningioma tissue shows cellularity and lack of pattern. (H&E X200).

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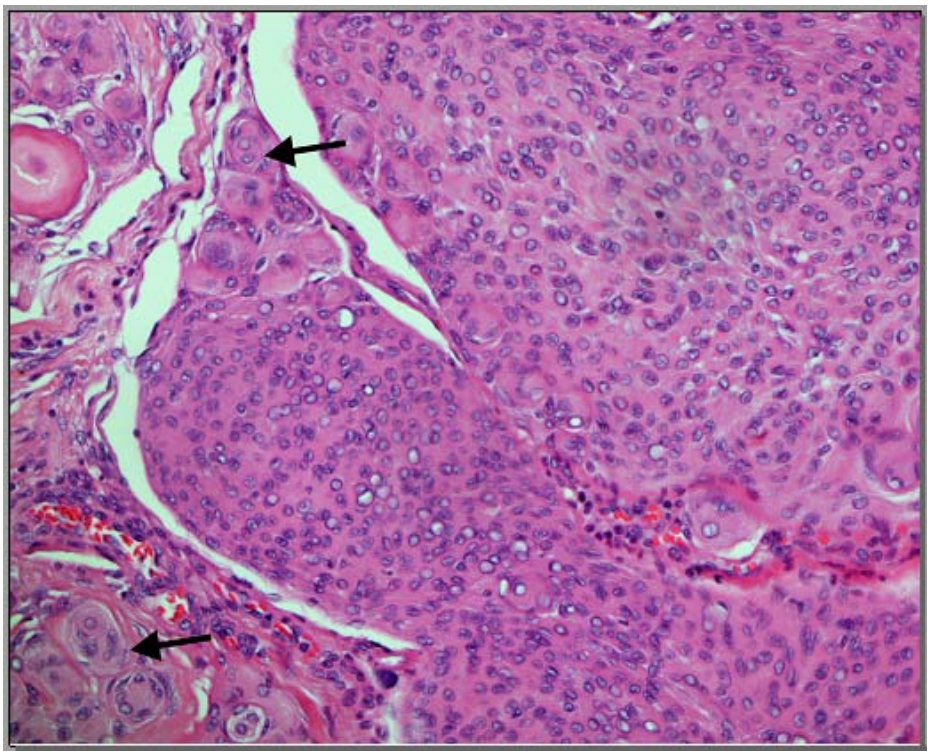


Figure 2: A meningioma showing cellular areas, foci of increased N: C ratio and partial lack of pattern. In the same field, meningothelial pattern with prominent whorls (arrows) is present (H&E X200).

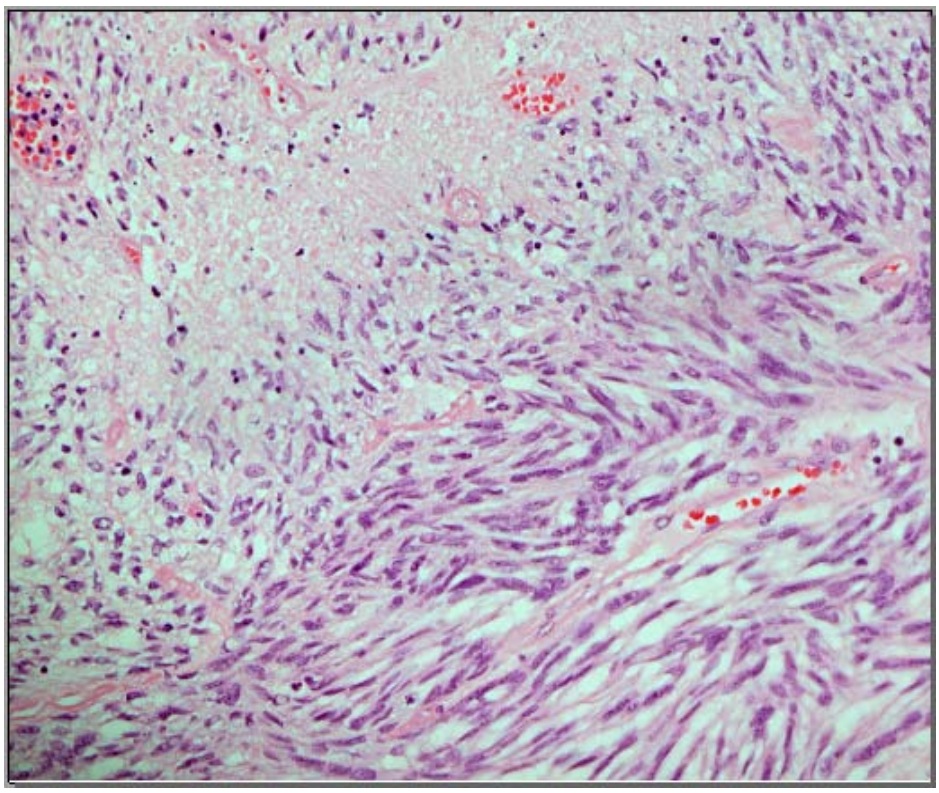


Figure 3: A meningioma showing focal necrosis and adjacent cellular proliferation of spindle cells with increased N: C ratio and foci of small cell change. Two pathologists considered such foci to be "sarcomatoid" (H&E, x200).

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Origanum vulgare (Oregano) and its Carvacrol Biocomponent as an Alternative of Antimicrobial Agent

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Abstract- The use of plants as an alternative to medicinal treatments is an old practice. The increased resistance of microorganisms to conventional antimicrobials has made studies with medicinal plants increasingly relevant, and ethnobotanical and ethnopharmacological knowledge is considered essential for the development of new drugs. The essential oil of Origanum vulgare and its isolated compound Carvacrol have antimicrobial effects demonstrated in the literature as antibacterial and antifungal activity. Therefore, the present study evaluated the antibacterial and antifungal activity of O. vulgare and Carvacrol using the broth microdilution method (CLSI, 2008), determining MIC (Minimum Inhibitory Concentration) and MFC and MBC (Minimum Fungicidal Concentration and Concentration Minimum Bactericide). Used as standard comparative the antimicrobials Fluconazoleand Chlorhexidine.

Keywords: *origanum vulgare; carvacrol; candida spp., streptococcus spp., staphylococcusaureus MRSA.*

GJMR-C Classification: *NLMC Code: QW 4*

ORIGANUMVULGAREOREGANDANDITSCARVACROLBIOCOMPONENTASANALTERNATIVEOFANTIMICROBIALAGENT

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Origanum vulgare (Oregano) and its Carvacrol Biocomponent as an Alternative of Antimicrobial Agent

Natália Kaori Aida [α], Janaina Priscila Barbosa [σ], Thaís Rossini de Oliveira [ρ], Vanessa da Silva Cardoso [ω], Simone Nataly Busato de Feiria [¥], Giovana Cláudia Boni [§] & José Francisco Höfling [χ]

Abstract- The use of plants as an alternative to medicinal treatments is an old practice. The increased resistance of microorganisms to conventional antimicrobials has made studies with medicinal plants increasingly relevant, and ethnobotanical and ethnopharmacological knowledge is considered essential for the development of new drugs. The essential oil of *Origanum vulgare* and its isolated compound Carvacrol have antimicrobial effects demonstrated in the literature as antibacterial and antifungal activity. Therefore, the present study evaluated the antibacterial and antifungal activity of *O. vulgare* and Carvacrol using the broth microdilution method (CLSI, 2008), determining MIC (Minimum Inhibitory Concentration) and MFC and MBC (Minimum Fungicidal Concentration and Concentration Minimum Bactericide). Used as standard comparative the antimicrobials Fluconazoleand Chlorhexidine. The essential oil of *Origanum vulgare* and the isolated compound Carvacrol are biologically active against the genders *Candida* and *Streptococcus* and in the strain of *Staphylococcus aureus* methicillin resistant, exhibiting promising antimicrobial properties. The antimicrobial activity can be seen highlighted with a combination of traditional commercial antimicrobials.

Keywords: origanum vulgare; carvacrol; candida spp., streptococcus spp., staphylococcusaureus MRSA.

I. Introduction

The use of plants as an alternative to medicinal treatments is an ancient practice, which provide primary health care to 80% of the world's population. It has also been an important source for new drug discoveries (Wangchuk & Tobgay, 2015). World Health Organization has estimated that around 80% of the population of developing countries use traditional herbal medicines as a source of treatment for diseases as a cheap and alternative source, other factors such as the lack of modern health facilities, Cultural priorities and choices also contribute to the use of medicinal plants as a therapeutic alternative (Aziz et al., 2018). Studies on medicinal plants have became increasingly relevant, ethnobotanical and ethnopharmacological knowledge is considered essential for the development of new drugs. Researches studies the effectiveness and use of traditional plants as alternative medicine and adjuvants in a treatment (Amjad et al., 2017). In this context, *Origanum vulgare* is a plantused since ancient times as a traditional cure for treating infections (Karaman et al., 2017). Studies show that *O. vulgare* essential oil is rich in Carvacrol, has anti-inflammatory, antioxidant, antispasmodic, antimicrobial, and antifungal activity (Król et al., 2019). Also, resistant microorganisms have emerged over the years and have been considered a global health threat. Microbial resistance has brought problems in the treatment of infectious diseases, and the development of new antimicrobial agents is required (Ayaz et al., 2019). The occurrence that strains of the genus *Candida* have been resistant to treatment with commercial antifungals, belonging to the azoles and polyenes family, has been a reason for concern by health professionals. *Candida* infections mainly affect immunosuppressed patients (Barbosa et al., 2019). Given this scenario, the plants are promising antimicrobial agent for the development of new drugs, acting in disease prevention and treatment (Ayaz et al., 2019). Therefore, this work aimed to study the antimicrobial activity of *Origanum vulgare* essential oil and its isolated compound Carvacrol against microorganisms of genus *Candida, Streptococci* and, *Staphylococci.*

II. Material and Methods

a) *Essential oil*

The essential oil was purchased commercially from Quinarí Fragrâncias e Cosméticos LTDA and the isolated compound from Sigma Aldrich.
Essential Oil - Lot: 09818DIV
Isolated Compound - Lot: S40656V

b) *Minimum Inhibitory Concentration Assessment (MIC)*

To determine the minimum inhibitory concentration (MIC), we tested samples of the essential oil and isolated compound against *Streptococcus* spp., *Candida* spp. and *Staphylococcus aureus* MRSA by broth microdilution technique following the M27-A3 protocol (CLSI, 2008).

c) *Candida spp. Inoculum adjustment*

It was prepared with saline in spectrophotometer an absorbance of 530nm in the

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range of 0.08 to 0.1 equivalent to 5.0 x 10^6 CFU/mL. Then, the inoculum was standardized, diluting to 2.5x 10^3, according to CLSI, 2008.

In asterile microplate was deposited with 100 µl of RPMI, 100 µl of the essential oil or 100 µl of the isolated compound at the initial concentration, followed by serial microdilution and then added with 100 µl of the adjusted inoculum.

Groups of test: CG: positive control group; OL: Essential Oil Treatment Group; IC: Treatment group with isolated compound; AC: Treatment group with the commercial antifungal Fluconazole at an initial concentration of 64ug/mL (CLSI, 2002). Incubated the plates for 48h at 37°C. Defined the MIC as the lowest concentration of compound that did not exhibit visible growth of the microorganism.

d) *Inoculum adjustment of the Streptococcus spp. and Staphylococcus aureus MRSA*

After growth in BHI liquid culture medium, the inoculum was adjusted by spectrophotometer with a wavelength of 625nm in the range 0.08 to 0.1absorbance, equivalent to 1.0 x 10^8 CFU/mL.

In a sterile microplate were deposited 100 µl of BHI, 100 µl of the essential oil or 100 µl of the isolated compound at the initial concentration, followed by serial microdilution, and then added of 100µl of the adjusted inoculum.

Groups of test: CG: positive control group; OL: Essential Oil Treatment Group; IC: Treatment group with isolated compound; AC: Treatment group with the commercial antifungal chlorhexidine at the initial concentration of 64ug/mL (CLSI, 2008). Incubation of the plates was made for 24h at 37°C with 10% CO_2 for *Streptococcus* strains and aerobically for *Staphylococcus aureus* MRSA. Defined the MIC as the lowest concentration of compound that did not exhibit visible growth of the microorganism.

e) *Determination of Minimum Fungicidal Concentration (CFM) and Minimum Bactericidal Concentration (CBM)*

In a petri dish containing Sabouraud Dextrose Agar (SDA) for yeast and Brain Heart Infusion Agar (BHI) for bacteria tested the determination of minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC). Homogeinized the wells containing target concentrations and transferred the aliquot to the Petri dish with the solid culture medium. After incubation at 37°C for 24h, established the lowest fungicidal/bactericidal concentration. Determined the MFC/MBC as the lowest concentration of essential oils and isolated compounds that did not allow the growth of any colony of the microorganism on the solid medium after the incubation period. Through visual reading, the inhibition of growth or death provided by the tested substances was confirmed (GULLO et al., 2012).

III. Results

a) *Evaluation of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal / Bactericidal Concentration (CFM / CBM)*

Tested the *Origanum vulgare* essential oil and its isolated compound Carvacrol against reference strains of the genus *Candida*, *Streptococcus,* and *Staphylococcus aureus* methicillin-resistant strain to determine its inhibitory effect by broth microdilution technique. Both substances showed inhibitory activity on microbial cells (Table 1).For reference strains of the genus *Candida,* inhibitory concentrations were between 0.125 mg/mL and 0.5 mg/mL for *O. vulgare* essential oil and 0.125 mg/mL and 0.0625 mg/mL for Carvacrol isolated compound. The antifungal Fluconazole was also tested against strains to determine the minimum inhibitory concentration by broth microdilution technique. Fluconazole has shown inhibitory activity between 1 and 32 µg/ml (Table 1). For bacterial strains of the genus *Streptococcus*, the essential oil of *O. vulgare* behaved similarly, varying the MIC between concentrations of 0.5 mg/mL and 0.250 mg/mL, as well as its isolated compound Carvacrol. The essential oil and Carvacrol inhibited the strain *Staphylococcus aureus* at concentrations of 0.5 and 0.250 mg/mL respectively. Chlorhexidine antimicrobial was also tested against strains to determine the minimum inhibitory concentration by broth microdilution technique. Chlorhexidine demonstrated inhibitory activity between 3.75 to 7 µg/ml (Table 1).After the determined the MIC values, used an aliquot of the susceptibility test to determine the minimum fungicidal/bactericidal concentration (MFC/MBC) of the strains. For *Candida* spp. Strains, *O. vulgare* essential oil, showed fungicidal activity against *Candida* spp. strains, varying its effect on concentrations between 0.5 mg/mL and 0.250 mg/mL; while Carvacrol showed 0.250 mg/mL fungicidal activity for all *Candida* strains tested (Table 1). *Streptococcus* bacterial strains showed a bactericidal concentration ranging from 0.5 mg/mL to 0.250 mg/mL for the essential oil and ranged from 0.5 mg/mL to 0.125 mg/mL for the isolated compound. *S. aureus*(MRSA) showed a bactericidal concentration of 0.5 mg/mL for both.

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Table 1: Visual reading results of MIC and CFM/CBM of the strains tested.

Reference strain	*O. vulgare*		Carvacrol		Fluconazole / Chlorhexidine
	MIC	MFC	MIC	MFC	
	mg/mL	mg/mL	mg/mL	mg/mL	µg/mL
C. albicans	0.25	0.5	0.125	0.25	1
C. dubliniensis	0.125	0.5	0.0625	0.25	1
C. glabrata	0.125	0.5	0.0625	0.25	1
C. parapsilosis	0.125	0.25	0.0625	0.25	2
C. krusei	0.25	0.5	0.125	0.25	32
C. guilliermondii	0.125	0.25	0.0625	0.25	1
S. mutans	0.5	0.5	0.25	0.5	3.75
S. mitis	0.25	0.25	0.125	0.125	3.75
S. oralis	0.5	0.5	0.25	0.25	15
S. gordonii	0.25	0.25	0.125	0.125	7.5
S. salivarius	0.25	0.5	0.25	0.25	3.75
S. sanguinis	0.25	0.5	0.25	0.25	7.5
***S aureus* MRSA**	0.5	0.5	0.25	0.5	

IV. Discussion

Excessive and indiscriminate use of antimicrobials is a major determinant of some emerging infections, selection of resistant pathogens, and the continued development of antimicrobial resistance globally. The increasing emergence of multi-drug resistant organisms and the limited development of new agents available to combat them have caused an imminent crisis with alarming implications (Sartelli et al., 2016).In view of the increasing numbers of cases of conventional drug-resistant microorganisms, researchers are looking for alternatives to biocompounds that have antimicrobial properties against microorganisms. Studies with plants as promising agents in the search for new compounds.

In this study, the obtained data showed antimicrobial activity of the tested essential oil, as well as its isolated compound against planktonic cells of *Candida* spp., oral *Streptococcus* species, and *S. aureus* methicillin resistant strain. The essential oil inhibited antimicrobial growth between concentrations of 0.5 to 0.125mg/mL against all strains tested. At the same time, the isolated compound showed antimicrobial activity between concentrations of 0.250 to 0.0625mg/mL against all strains tested. The minimum fungicidal/bactericidal concentration (MFC/MBC) of the essential oil in the strains tested was between 0.5 mg / ml and 0.250 mg / ml. The isolated compound showed MFC/MBC between concentrations of0.5 mg/mL to 0.125 mg/mL (Table 1).

These data, initially reveal the antimicrobial action of this essential oil, as well as its isolated compound, corroborating with the literature, pointing out its antimicrobial activity. In a study by Bharti et al. (2013). *O. vulgare* essential oil also demonstrated antimicrobial activity in synergism with ciprofloxacin against clinical isolates of *Salmonella typhi*, considerably decreasing the inhibitory concentration of conventional antimicrobial. According to the study by Bhat et al. (2018), antifungal activity demonstrated an inhibition zone of 30 mm for *O. vulgare* compared with 22 mm for nystatin against the three *Candida* species tested, *Candida glabrata, Candida tropicalis,* and *Candida albicans*. Cleff et al. (2010) also observed the antifungal activity of *O. vulgare* against *C. albicans, C. parapsilosis, C. krusei, C. lusitaniae,* and *C. dubliniensis* strains, and in this same study, the action of the essential oil against isolates was also tested. Clinical results of *C. albicans* showed dose-dependent antifungal activity for the strains tested. The mechanism of Carvacrol action was investigated by Wang et al. (2016) showing that exposure to Carvacrol at low concentrations induced a marked increase in unbranched fatty acid content and at higher levels substantially altered the integrity and morphology of *S. aureus* cell membrane.

Nobrega et al. (2016) evaluated the minimum inhibitory concentration and the minimum fungicidal concentration of Carvacrol, ranging from 25 to 81 µg/mL MIC and 25 to 102 µg/mL CFM. According to Duarte et al. (2005), the MIC value is parameter of the classification of the acceptance level of plant materials, up to 0.5 mg/mL being considered strong, from 0.55 to 1.5 mg/mL, moderate and above. 1.5 mg/mL as weak. In this sense, the results obtained with *O. vulgare* essential oil and isolated compound showed MICs considered strong for all strains tested.

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The data obtained in this study added to the data in the recent literature, suggest that the essential oil of *O. vulgare* and also its isolated compound Carvacrol show antibacterial and antifungal potential.

These data open possibilities for many other studies, such as the performance of these oils and biocomponents in mature biofilms and multispecies biofilms, evaluating cell viability and possible morphological changes, added to cytotoxicity tests, action on cancer cells, in an attempt to add more information about this plant and its use as an alternative agent in the treatment of infections and acting as coadjuvants.

V. Conclusion

- Both the essential oil of *O. vulgare*, and its isolated compound Carvacrol, can inhibit the growth of the tested microorganisms in low concentrations;
- The isolated compound is more effective when compared to the essential oil, inhibiting the microorganisms in a lower concentration;
- About the microorganisms, essential oil and Carvacrol are more effective against *Candida* spp. when compared to bacterial strains;
- Both the EO and its main biocomponent Carvacrol show fungicidal/bactericidal activity against the tested strains

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Effects of Antimicrobial Application of Greencop-Pro1, Greencop-Pro2 and Nano-Aq on Chick Quality in the Incubation Period of Japanese Quail (Coturnix Coturnix Japonica)

By Emre Aydemir

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Abstract- World-wide poultry production is increasing day by day. One of the problems encountered in poultry production is disinfection. There are many microorganisms such as bacteria, viruses, fungi and parasites in the incubators. These microorganisms have negative effects such as the incubation of eggs incubated in the embryonic period, low chick weight, and poor chick quality. These effects lead to significant economic losses in commercial production. There are many disinfection applications to prevent these economic losses. In this study, the effects of antimicrobials on Japanese quail eggs in the embryonic period were investigated. The study control group consists of the 4th group as Greencop-Pro1, Greencop-Pro2 and Nano-Aq. There are 100 eggs for each group and 400 eggs for recurrence.In the study, a total of 1200 Japanese quails were used for 3 recurrences. For each antimicrobial 1 liter, 25 mg / kg x 5 was diluted to 25 mg / kg.

Keywords: *chick quality, antimicrobial, greencop-pro1, greencop-pro2, nano-aq.*

GJMR-C Classification: *NLMC Code: QV 325*

EFFECTSOFANTIMICROBIALAPPLICATIONOFGREENCOPPRO1GREENCOPPRO2ANDNANOAQONCHICKQUALITYINTHEINCUBATIONPERIODOFJAPANESEQUAILCOTURNIXCOTURNIXJAPONICA

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Effects of Antimicrobial Application of Greencop-Pro1, Greencop-Pro2 and Nano-Aq on Chick Quality in the Incubation Period of Japanese Quail (Coturnix Coturnix Japonica)

Emre Aydemir

Abstract- World-wide poultry production is increasing day by day. One of the problems encountered in poultry production is disinfection. There are many microorganisms such as bacteria, viruses, fungi and parasites in the incubators. These microorganisms have negative effects such as the incubation of eggs incubated in the embryonic period, low chick weight, and poor chick quality. These effects lead to significant economic losses in commercial production. There are many disinfection applications to prevent these economic losses. In this study, the effects of antimicrobials on Japanese quail eggs in the embryonic period were investigated. The study control group consists of the 4th group as Greencop-Pro1, Greencop-Pro2 and Nano-Aq. There are 100 eggs for each group and 400 eggs for recurrence.In the study, a total of 1200 Japanese quails were used for 3 recurrences. For each antimicrobial 1 liter, 25 mg / kg x 5 was diluted to 25 mg / kg. In the study, the chick quality obtained from each incubator was determined using the Tona Score method. Duncan and Tukey multiple comparison tests were performed for the parametric test to determine whether there was a difference between the study. Games Howell test test was applied for non-parametric groups. When the results obtained were examined, it was statistically estimated that the incubation efficiency of the group with Nano-Aq antimictobial application was higher and the chick quality was better.

Keywords: chick quality, antimicrobial, greencop-pro1, greencop-pro2, nano-aq.

I. Introduction

Due to the developments in poultry farmingworldwide, poultry meat production has increased significantly in the last 30 years. When world meat production is examinedtoday, 37.27% of the production amount is provided from chicken, whilepork meat is produced by 36.52%, cattle meat by 21.69% and ovine meat by 4.51% (FAO, 2018). While the total amount of meat producedwas330.5milliontons in 2018, FAO announcedthat the total amount of meat will reach 357.5 milliontons in 2025, it is estimatedthat the amount of poultry meat will have the highest share in this production share. In particular, in the world chicken meat production in the United States, Brazil, China, European Union, India, Russia, Mexico, Argentina, Turkey, Thailand, Indonesia is located in the first row (USDA-FAS 2018). In addition to chicken meat production worldwide, the consumption of small species such as turkey, goose, duck, partridge and quail has increased significantly in recent years.It occurs in various problems with the increase in production significantly. These problemsincludepoorcleaning of the incubator, tools and equipment (Avens et al., 1974; Whistler and Sheldon, 1989,; Brake and Sheldon, 1991).There are various microorganisms such as bacteria, viruses, fungi and parasites on them. The medium in the embryo has the necessary conditions for the growth of microorganisms. Under unfavorable conditions during incubation, embryo development is prevented. Accordingly, it has negative effects on chick quality, embryonic deaths, growth and development. In addition, economic losses increase due to losses (Sacco et al., 1989; Scott and Swetnam, 1993, Reid et al., 1961). To providedisinfection during the incubation period; materials such as fumigation, UV light, spray, various organicacids, vinegar, antimicrobial and antibacterial are used. (Adler et al. 1979; Arhienbuwa et al. 1980; Kuhl, 1989; Proudfoot et al., 1985; Sacco et al., 1989; Whistler &Sheldon, 1989).Preventing the formation of microorganisms such as bacteria, viruses, fungi and parasites in incubation causesboth the decrease in embryonic mortality rate and the increase of chick quality (Scott and Swetnam, 1993; Sacco et al. 1989; Reid et al. 1961).

a) Some Studies Done Worldwide

It was for Fren and Sheldon, (1990) to apply different doses of couverternar ammonium (1.05% and 3%) to eggs obtained from flocks of five different ages (32, 36, 42, 46, 62 weeks).In the study, Brake and Sheldon (1990) stated that the application of couverternar ammonium increased hatching efficiency by 6% in eggs. In a study using turkey derived eggs used as model animal, Sacco et al. (1989) observed the effects of quaternary ammonium compounds and formalin fumigation on shell antimicrobial activity, efficacy and embryonic survival. Sacco et al. (1989) stated that embryonic viability of the group in which the application of couverternar ammonium was applied in 2

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trials differed statistically (P <0.05).They also reported that there was no significant difference in antimicrobial activities in the third trial group.In a study by Shahein and Sedeek (2014), they observed the effects of 7% and 14% propolis, 0.5% and 0.7% thyme essential oil, 70% ethyl alcohol, formaldehyde and control group.In the study, the number of chicks obtained from 14% propolis application was higher than the other groups; reported that embryonic mortality rates were at least 7% and 14% in propolis-treated groups. In the study where Japanese quail was used as a model animal, Fouad et al. (2018) used garlic oil as a disinfectant. In the study, they observed that the application of 1ml / liter and 2ml / liter of garlic oil solution was significantly higher in hatching efficiency, chick weight, and chick length compared to the control group (P <0.05). Fouad et al. (2019) in their study, they observed the differences between the control group and the hatching efficiency of the vinegar applied in 3 different doses (1.25%, 2.5 and 5) as disinfectant. In the study, they stated that the vinegar they applied as a spray was statistically more embryo weight, chick weight and length (P <0.05).In a similar study, Manwar et al. (2012) reported that vinegar application increased chick weight. In another similar study, they stated that the application of vinegar as disinfectant has effects on embryo development, egg weight, gas exchange, metabolism and development (Paganelli et al., 1978; Rahn et al., 1979, Rahn and Ar, 1980 and Burton and Tullet, 1983).In a study by Debes and Basyony (2011), they examined the effects of thyme (Origanum vulgarel) and ginger (Zingiber officinale) oil on White Leghorn and Matrouh chicken eggs. When the incubation efficiency was examined in the study, 86.45% in the control group, 89.46% in formaldehyde, 87.08% in alcohol group, 94.40% in thyme oil, they stated that it was 93.66% in ginger oil and 94.96 ± 0.266% in thyme and ginger mixture. They also reported that the application of thyme and ginger oil reduced embryo mortality, increased chick weight and had a positive effect on performance. Batkowska et al. (2018) used red grapefruit juice as a disinfectant for Japanese quail eggs. First group control group in the study, group 2 formaldehyde and KMnO4 and red grapefruit juice was applied to the third group. In the study results, they stated that using red grapefruit juice as a disinfectant had no effect. In their study, Marlina et al. (2017) used three different amounts (25%, 50%, 75%) as antibacterial disinfection of guava leaf water. In the study results, they stated that the use of 75% guava leaf water decreased the total number of bacteria by 89.53%.

b) Determination of Chick Quality

A quality chick shouldpossess the characteristics of optimum development during incubation, high survival, good growth after emergence, and efficiency in accordance with standards. The eyes of a quality chick thatcomes out of the incubation and driesshould be bright, without anydeformity or wound in the body, the belly is completelyclosed, the yellow is completelyremoved, and it is free from the membrane and shell residues. This chicks should be able to give a reaction, thereshould be no edema, lesion or similarswelling in the body, it shouldreact to externalsounds or different stimuli, be awake and activelyrelated to itsenvironment (Tona et al., 2005).Considering all these features, chicks are divided into different classes according to physical features (Tona et al., 2004, 2003a, 2003b, 2001).

c) Tona Score Method

The general activity and appearance of chicks of a day old age that has justhatched and dried out the tonna score, the presence and amount of yellowresidue, the condition of the eyes, navel area and legs, the presence and quantity of hered membranes, ıt is a qualitative method that is evaluated over 100 pointsconsidering the egg yolk withdrawal criteria.The quality criteriondecreases for an abnormality in eachcriterionconsidered. Also performance, efficiency, etc. It helps to estimate the criteria (Tona et al. 2003).

Table1.1: Criteria for Determining Chick Quality in Tona Score Method

Quality criterion	Determination Conditions	Score
Activity	Activity is assessed by laying the chick on itsback to determine how quickly it returned to itsfeet. A quick spring back on to its feet was regarded as good, but trailing back on to its feet or remaining on its back was assessed as weak.	6-0
Down and appearance	The chick body wasexamined for dryness and cleanness. It wasregarded as normal if it is dry and clean. If it is wet or dirty or boththen it is not good.	10-8-0
Retracted yolk	The chick was put on itsbackobliquely on the handpalmuntil abdominal movement totally stopped. The height of its abdomen wasestimated. The consistency of the abdomen to touch was then estimated. If the height of abdomen was estimated to be higher and harder to touchthan normal, then yolk retracted was regarded as large and consistent.	16-12-8-4-0

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Eyes	The chick was put on the legs, and its eyes were observed. The state of brightness and wideness of the gape of the eyelids were estimated.	16-8-0
Legs	The chick was put on itsfeet to determine if it remaine dupright well. The toes were examined for their conformation. If the chick remaine dupright with difficulty, articulations of the knees were examined to detect signs of inflammation or redness or both.	16-8-0
Navel area	Navel and surrounding are as were examined for closure of the navel and its coloration. If the colorwas different from the skin color of the chick, then it was regarded as bad.	12-8-4-0
Remaining membrane	Observation of the navel area allowed estimation of the size of any remaining membrane. The size of any remaining membrane was classified as very large, large, or small.	12-6-0
Remaining yolk	Observation of the navel area allowed estimation of the size of any remaining yolk. The size of any remaining yolk was classified as very large, large, or small.	12-0

(Tona et.al., 2003).

II. Material and Method

In the study, 4 experiment groups, Greencop-Pro1, Greencop-Pro2, Nano-Aq and controlgroup were used. A separate incubator was used for each trial group. A total of 400 Japanese quail eggs, 100 of which were included in each incubator, were placed. A total of 1200 Japanese quail eggs were used, 400 for each recurrence. Greencop-Pro1, Greencop-Pro2 and Nano-Aq used antimicrobially in the study were systematically adjusted by automated sprays during the incubation period. For each antimicrobial 1 liter, 25 mg / kg was diluted in the amount of 25 mg / kg x 5. In the study, the chick quality obtained from each incubator was determined using the Tona Score method. In addition, the incubation efficiency was examined for eachgroup. In addition, the total number of chicks obtained from the eggs laid for each incubator was also examined.

For the variables that meet the parametric test assumptions for the statistical analysis of the data obtained from the study, it was revealed whether there is a difference between the variance analysis technique and the experiment groups at the level of 5% significance. All statistical analyzes were done using SPSS statistical software. Variance analysis technique for variables has been demonstrated with anova test whether there is a difference between experiment groups and 5% significance level. Duncan and Tukey multiple comparison tests were conducted for the parametric test to determine which group or groups originated from the differences. For non-parametric groups, Games Howell test test was applied.

III. Results

In the study, when the incubation efficiencies were examined in the first recurrence Greencop-Pro1 84%, Greencop-Pro2 91%, Nano-Aq 97% and control group 87% determined to be.In the secondrecurrence, it wasobservedthat the incubation efficiencywas 90%, 86%, 94% and 83% in the same order. In the thirdrecurrence, 85%, 87%, 96% and 86% were determined in the same order. In the study, it was found that the highest incubation efficiency among the groupswas in the group with Nano-Aq antimicrobial application (Table1.1.). When recurrence group average was examined, it wasfound as 89.75, 88.25 and 88.5. Of these mean values, Nano-Aq antimicrobial applied group was estimated to have a statistically significant difference in hatching efficiency (Table 1.1.).

In the second recurrence, it wasobservedthat the incubation efficiencywas 90%, 86%, 94% and 83% in the same order.In the third recurrence, 85%, 87%, 96% and 86% were determined in the same order.In the study, it wasfoundthat the highest incubation efficiency among the groups was in the group with Nano-Aq antimicrobial application (Table1.1.). When recurrences group average was examined, it wasfound as 89.75, 88.25 and 88.5. Of these mean values, Nano-Aq antimicrobial applied group was estimated to have a statistically significant difference in hatching efficiency (Table 1.1.).

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Tablo 1.1: Number of chicks obtained from Greencop-Pro1 Greencop-Pro2 Nano-Aq and Control Groups

	Greencop-Pro1	Greencop-Pro2	Nano-Aq	Control Group	Groupsmean
		1st recurrence			
Number of eggs	100	100	100	100	100
Number of chicks	84	91	97	87	89.75
		2 strecurrence			
Number of eggs	100	100	100	100	100
Number of chicks	90	86	94	83	88.25
		3 strecurrence			
Number of eggs	100	100	100	100	100
Number of chicks	85	87	96	86	88.5

When the chick quality for the first recurrence was examined in the study, Greencop-Pro1 Greencop-Pro2 Nano-Aq and Tona Score scores for the control group were; It was found as 93.6, 97.29, 99.85 and 93.27 (Table 1.2.).It was determined that the group with the highest chick quality among the groups with antimicrobial application was in the group with Nano-Aq. When the second recurrence is examined, Tona Score scores are in the same order; The grooves 95.15, 93.86, 99.2 and 92.42were estimated. For the third recurrence, Tona is for Score scores; Designed as 96.68, 95.27, 98.6 and 93.86(Table 1.2.)

Tablo 1.2: Of Greencop-Pro1 Greencop-Pro2 Nano-Aq and Control Groups' Tona Score and Pasgar Score.

	Greencop-Pro1	Greencop-Pro2	Nano-Aq	Control Group	Groupsmean
			1st recurrence		
Tona Skor Mean	93.6	97.29	99.85	93.27	93.86
			2 strecurrence		
Tona Skor Mean	95.15	93.86	99.2	92.42	95.15
			3 strecurrence		
Tona Skor Mean	96.68	95.27	98.6	93.86	96.01

When the results obtained in the study are analyzed, Nano-Aq antimicrobial application group; Tona Score score was higher than other groups. Nano-Aq antimicrobial administration has affected the chick quality positively in the incubation period. When the total number of chicks hatched from the egg was examined, it was observed that the incubation efficiency of the group with Nano-Aq antimicrobial application was highest.

IV. Conclusions

In the study, when the incubation efficiency was examined for Greencop-Pro1, Greencop-Pro2, Nano-Aq and antimicrobial applications, it was observed that Nano-Aq antimicrobial application increased the incubation efficiency in the 1st, 2nd and 3rd recurrences. In the study, when eggs were examined after incubation, it was found that early and late embryonic deaths were highest in the control group. When the chick quality was examined in the study, the lowest (93.27, 93.27, 92.42) value in all three recurrences belonged to the control group; the highest (99.85, 99.2, 98.6) value was found to be in the group with Nano-Aq application. Chick quality defects; The foot problem was observed that the navel area was not closed and the yellow sac was not pulled in. The group with the most effective results in Greencop-Pro1, Greencop-Pro2, Nano-Aq and antimicrobial applications applied in the study was determined as the group that applied Nano-Aq.

Nano-Aq content feature is bacteria, virus, fungus and parasite. The effective feature of Greencop-Pro1 content is bacteria. The effective feature of Greencop-Pro2 content is on mushrooms. Since the applied antimictobials are commercial products, content information Ertuğrul ARPAÇ belongs to.

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Compositional Analysis, Antioxidant and Antimicrobial Potential of the Seed Extract of *Annona cinerea* Dunal Grown in Nigeria

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Abstract- Seed of *Annona cinerea* grown in Nigeria was investigated for its secondary metabolites and antioxidant potential using Gas Chromatography-Mass Spectrometry (GC-MS), 2,2'-dyphenyl-1-picrylhydrazyl (DPPH) and 2,6-ditert-butyl-4-[(3,5-ditert-butyl-4-λ1-oxidanylphenyl)methylidene] cyclohexa-2,5-dien-1-one (Galvinoxyl), respectively. The antibacterial activity of the seed extract was evaluated on eleven (11) pathogenic bacteria using agar well diffusion method at different concentrations of the extract. Twenty-seven (27) therapeutically active secondary metabolites were identified in the seed extract using GC-MS and the principal constituents identified were 3-O-methyl-d-glucose (52.14%), **β**-sitosterol (11.79%), desulphosinigrin (6.16%) and **α**-tocopherol (5.84%). The extract also displayed high DPPH and galvinoxyl radical scavenging activity with IC_{50} values of 5.0 and 100 μgml^{-1}. The zones of inhibition ranged from 10-30 mm against all tested bacteria. The antibacterial index (AI) ranged between 0.5-25. This study demonstrated that the seed of *A. cinerea* could be a potential source of natural antioxidants and antimicrobial agents.

Keywords: *Annona cinerea, phytochemical, free radical scavenging, antimicrobial activities.*

GJMR-C Classification: *NLMC Code: QV 325*



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Compositional Analysis, Antioxidant and Antimicrobial Potential of the Seed Extract of *Annona cinerea* Dunal Grown in Nigeria

Ololade, Zacchaeus S. [α] & Anuoluwa, Iyadunni A. [σ]

Abstract- Seed of *Annona cinerea* grown in Nigeria was investigated for its secondary metabolites and antioxidant potential using Gas Chromatography-Mass Spectrometry (GC-MS), 2,2′-dyphenyl-1-picrylhydrazyl (DPPH) and 2,6-ditert-butyl-4-[(3,5-ditert-butyl-4-λ1-oxidanylphenyl)methylidene]cycl-ohexa-2,5-dien-1-one (Galvinoxyl), respectively. The antibacterial activity of the seed extract was evaluated on eleven (11) pathogenic bacteria using agar well diffusion method at different concentrations of the extract. Twenty-seven (27) therapeutically active secondary metabolites were identified in the seed extract using GC-MS and the principal constituents identified were 3-O-methyl-d-glucose (52.14%), *β*-sitosterol (11.79%), desulphosinigrin (6.16%) and *α*-tocopherol (5.84%). The extract also displayed high DPPH and galvinoxyl radical scavenging activity with IC_{50} values of 5.0 and 100 μgml^{-1}. The zones of inhibition ranged from 10-30 mm against all tested bacteria. The antibacterial index (AI) ranged between 0.5-25. This study demonstrated that the seed of *A. cinerea* could be a potential source of natural antioxidants and antimicrobial agents.

Keywords: Annona cinerea, phytochemical, free radical scavenging, antimicrobial activities.

I. Introduction

Medicinal plants are of great importance to the health of humans. The medicinal value of green plants lies in the ability of some secondary metabolites to produce a definite physiological action in the human body (Dwivedi *et al*., 2017; Adusei *et al*., 2019; Haque *et al*., 2019). Our environment is richly blessed with enormous biodiversity of plants that can be used for both consumption and therapeutic purposes. Plants play remarkable roles in and contributes to human diets and food security (Bharucha and Pretty, 2010; Chandrasekara and Kumar, 2016; Chen *et al*., 2016). Unfortunately, oftentimes the utilisation and knowledge of medicinal plants as a nutritional source is confined to rural settlements (Aryal *et al*., 2019). Medicinal plants have been used globally to meet health care needs of human and animals. Recently, medicinal plants have witnessed a glut of research geared towards validating the quality, quantity, protective roles as well as therapeutic effectiveness of these natural antioxidants in medicinal plants against oxidative stress induced diseases and disorders (Lawal *et al*., 2016; Bourhia *et al*., 2019; Shaito *et al*., 2020). Presence of scientific literature on antioxidants and antimicrobial activity of phytochemicals to a great extent validates the traditional claims about the usefulness of these medicinal plants to treat reactive oxygen species (ROS) induced health related disorder (Liu *et al*., 2018; Forni *et al*., 2019; Khameneh *et al*., 2019). Free radicals such as reactive oxygen species (ROS) are usually produced as a result of an organism's normal use of oxygen. An imbalance between formation and removal of these free radicals can lead to a pathological condition called oxidative stress resulting in many physiological processes like aging and chronic diseases (Aprioku, 2013; Phaniendra *et al*., 2015). However, the human body employs antioxidants to counteract these free radicals thus repairing free radical damage by initiating cell regeneration or cell repair (Lobo *et al*., 2010; He *et al*., 2017; Pizzino *et al*., 2017). Daily consumption of natural products that are rich in antioxidants, such as vegetables and fruits play an important role in the prevention and treatment of oxidative stress-related diseases such as cancer, arthritis, liver injury, diabetes, Alzheimer's disease, cardiovascular problems, neurodegenerative disorders, and various inflammatory illnesses (Tan *et al*., 2018; Forni *et al*., 2019; Mattia *et al*., 2019). Incorporation of antioxidant compounds by consuming natural products in the daily diet can be a suitable solution to solving human health issues. These natural antioxidant sources can be used as a preventive medicine. Recent researches showed that there is an inverse link between the dietary consumption of antioxidant-rich foods and prevalence of human illness (Arulselvan *et al*., 2016; Wilson *et al*., 2017; Liu *et al*., 2018; Lourenco *et al*., 2019; Villaverde *et al*., 2019). Moreover, the use of natural products as antibiotic agents is been given more attention due to the various side effects and increasing antibiotic resistance to synthetic antibiotics observed in some pathogens responsible for food borne and other illnesses (Fair and Tor, 2014; Barbieri *et al*., 2017; Cheesman *et al*., 2017; Albaridi, 2019; Dilbato *et al*., 2019). Natural antimicrobials seems to be the most promising solution to many of the increasing concerns regarding antibiotic resistance and could yield better

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results than antimicrobials from combinatorial chemistry and other synthetic procedures (Rossiter *et al*., 2017; Armas *et al*., 2019; Tyers and Wright, 2019). Therefore, novel types of effective and healthy antimicrobial compounds that could protect food against contamination and consumer against infection is in high demand. Compounds derived from natural sources have the potential to be used for food safety due to their antimicrobial properties against a broad range of foodborne pathogens (Lucera *et al*., 2012; Hintz *et al*., 2015; Quinto *et al*., 2019).

Annona cinerea Dunal (*Annonaceae*) is a green perennial plant that annually produces edible fruit which have many medicinal advantages. It is a nutritional rich fruit and is largely valued for its taste. It is high in energy and is a good source of minerals such as iron, phosphorus and potassium. The fruit of the plant is high in energy and low in fat content, sodium content, free from cholesterol. It is also a good source of fibre, iron, potassium, phosphorus, manganese, copper, zinc, magnesium, vitamins B1, B2, B6 and C (Zahid *et al*., 2017; Sharma *et al*., 2019). It is known to have some active phytochemicals against the common chronic and degenerative diseases such as cancer, respiratory, neurodegenerative, and digestive diseases (Jammala *et al*., 2019; Singh *et al*., 2019).

To the best of our knowledge, there is no enough scientific information on the chemical composition and medicinal properties of seed of *A. cinerea* grown in Nigeria so far. Therefore, the present research was undertaken to screen extract of the seed of *A. cinerea* grown in Nigeria for its chemical composition, antioxidant and antimicrobial potentials.

II. Materials and Methods

a) Collection of Plant Sample

The plant material was collected in Ota, Ogun State, Nigeria and it was identified as *Annona cinerea* Dunal.

b) Preparation and Extraction of the Plant Sample

Air-dried and pulverised seed were soaked in a mixture of methanol/ethyl acetate (2:1). The mixture was left for at least three days. The filtrate was concentrated using a water bath. The concentrated extract was put into a vial and stored in a refrigerator to prevent contamination pending subsequent analysis (Emmanuel *et al*., 2014).

c) Gas Chromatography-Mass Spectroscopy Analysis of the Extract for Various Secondary Metabolites

The qualitative and quantitative analysis of the secondary metabolites in the extract was carried out using GC-MS QP2010 Plus (Shimadzu, Kyoto, Japan) system at the Shimadzu Training Centre for Analytical Instruments (STC) Lagos, Nigeria. The analytical specifications of the GC-MS were done as described in an earlier study (Ololade *et al*., 2014).

d) In vitro Antioxidant Activities

The antioxidant capacity of the seed extract of *A. cinerea* was tested using two different methods.

i. In vitro 2,2′-Diphenyl-1-picryl-hydrazyl Assay

The antioxidant and free radical scavenging of the extract of *A. cinerea* were measured by using 2, 2′-diphenyl-1-picryl-hydrazyl according to the method decribed by Lin *et al*., (2018) with minor modification. Briefly, the reaction mixture (2.0 ml) consists of 2.0 ml of 0.1 mM DPPH prepared by dissolving 4 mg of DPPH in 100ml of methanol and then 1.0 ml of various concentrations of the extract. It was incubated for 30 min. in the dark, and the absorbance was measured at 517 nm using SM 7504 UV Spectrophotometer. The blank contained a preparation of DPPH and methanol in place of extract. In this assay, the positive control was ascorbic acid. The percentage of the radical inhibition activity was evaluated based on the following expression:

$$I\%_{DPPH^{\bullet}} = \frac{A_{blank} - A_{ext}}{A_{blank}} X\ 100$$

Where: A_{blank} and A_{ext} are the absorbance value for the blank and extract solution, respectively. The dose-response curve was plotted and IC_{50} value for the extract and the standard were calculated.

ii. In vitro 2,6-ditert-butyl-4-[(3,5-ditert-butyl-4-λ1-oxidanylphenyl)methylidene]cyclohexa-2,5-dien-1-one (Galvinoxyl), respectively.

The antioxidant and free radical scavenging of the seed extract of *A. cinerea* were also evaluated using galvinoxyl according to the method previously described by Amira *et al*., (2012) with slight modification. Briefly, the reaction mixture with a total volume of 2.0 ml consists of 1.0 ml of 0.1mM Galvinoxyl which was prepared by dissolving 4.2 mg of Galvinoxyl in 100 ml of methanol and then 1.0 ml of various concentrations of the extract, was incubated for at least 30 min. in the dark, and then the absorbance was measured at 429 nm using SM 7504 UV Spectrophotometer. The blank was prepared by galvinoxyl and methanol in place of sample. In this assay, the positive control was ascorbic acid. The percentage of the radical inhibition activity was calculated based on the following expression:

$$I\%_{\text{Galvinoxyl}} = \frac{A_{blank} - A_{ext}}{A_{blank}} X\ 100$$

A_{blank} and A_{ext} are the absorbance value for the blank and extract solutions, respectively. The dose-response curve was plotted and IC_{50} value for the extract and the standard were calculated.

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Antioxidant Activity Index (AAI): The AAI was calculated as:

$$AAI = \frac{Galvinoxyl\ or\ DPPH^{\bullet} Initial\ Concentration}{IC_{50}}$$

AAI was classified as weak; when AAI < 0.5; moderate, when AAI ranged between 0.5-1.0; strong; when AAI ranged between 1.0-2.0; and very strong; when AAI > 2.0.

e) In vitro Screening of Antibacterial Potential

Antibacterial assay of the extract at different concentrations was performed using agar well diffusion assay on sterilized Mueller Hinton Agar (MHA) using streak plate method according to the method previously used by Debalke *et al*., (2018). Gram-positive bacteria used for the antibacterial test were *Bacillus sp*, *Enterococcus faecalis*, *Micrococcus varians* and *Streptococcus agalactiae* while the Gram-negative bacteria were *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Providencia stuartii*, *Salmonella typhimurium*, *Serratia marcescens* and *Shigella dysenteriae.* Cefuroxime (CRX) 30 µg/disc was used as positive control. After incubation for 18-24 hr at 37 °C, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZI) was observed and measured in millimetre (mm) using transparent ruler.

f) Determination of the Antibacterial Activity Index (AI)

The AI of the test seed extract with respect to the positive control was done according to the method previously used by Ololade *et al*., 2020.

III. Results and Discussion

a) Chemical Constituent of the Seed Extract of Annona cinerea

The total ion chromatogram (TIC) of the methanol/ethyl acetate seed extract, showing the GC-MS profile of the compounds identified is as shown in Figure 1. The peaks in the chromatogram were integrated and compared with the database of spectrum of known components stored in the GC-MS NIST library. Phytochemical screening by GC-MS analysis of the seed extract of *A. cinerea* revealed the presence of different classes of organic compounds. A total of twenty-seven (27) phytochemicals were identified in the seed extract accounting for 99.45% of the extract (Table 1), and the main constituents identified were 3-O-methyl-d-glucose (52.14%), ß-sitosterol (11.79%), desulphosinigrin (6.16%) and α-tocopherol (5.84%). Previous studies on the chemical composition of leaf extract of *A. muricata* from Uganda showed the presence of Z-7-tetradecenal (9.39%), n-hexadecanoic acid (7.12%), oleryl alcohol (6.15%), phytol (5.61%) as its main constituents (Gavamukulya *et al*., 2015). 3-O-methyl-d-glucose is used as a marker to assess glucose transport by evaluating its uptake within various cells and organ systems.

Table 1: Chemical Composition of the Seed Extract of *Annona cinerea*

Compound	Retention Index	Percentage Composition
6-oxa-bicyclo[3.1.0]hexan-3-one	782	0.97
4,4-dimethyl-2-pentanol	795	0.1
isopropylmethylnitrosamine	813	0.62
(S)-(+)-2-amino-3-methyl-1-butanol	876	1.06
α-furylcarbinol	885	0.43
2-hydroxy-γ-butyrolactone	1013	1.21
2,5-dimethyl-4-hydroxy-3(2H)-furanone	1022	0.59
3-methyl-3-cyclohexen-1-carboxaldehyde	1041	0.49
N,N-dimethyl(1H-pyrrol-3-yl)methanamine	1047	0.25
3-heptenoicacid	1081	0.53
3,5,5-trimethylhexanoicacid	1124	0.1
levomenthol	1144	0.48
1-trimethylsilyloxy-2-cyclohexylethane	1164	3.84
2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	1173	0.26
5-amino-3-methylisoxazole-4-carbonitrile	1248	0.26
3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	1269	1.78
2-(2-butoxyethoxy)aceticacid	1325	0.1
1,3:2,5-dimethylene-l-rhamnitol	1426	1.0
9,9-dimethoxybicyclo[3.3.1]nona-2,4-dione	1610	0.39

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3-O-methyl-d-glucose	1648	52.14
desulphosinigrin	2509	6.16
β-sitosterol	2731	11.79
α-tocopherol	3149	5.84
25-[(trimethylsilyl)oxy]-(3β,5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-1,3-diol	3258	4.09
adenosine,N6-phenylaceticacid	3731	1.0
stevioside	6530	3.97
Percentage Total		**99.45**

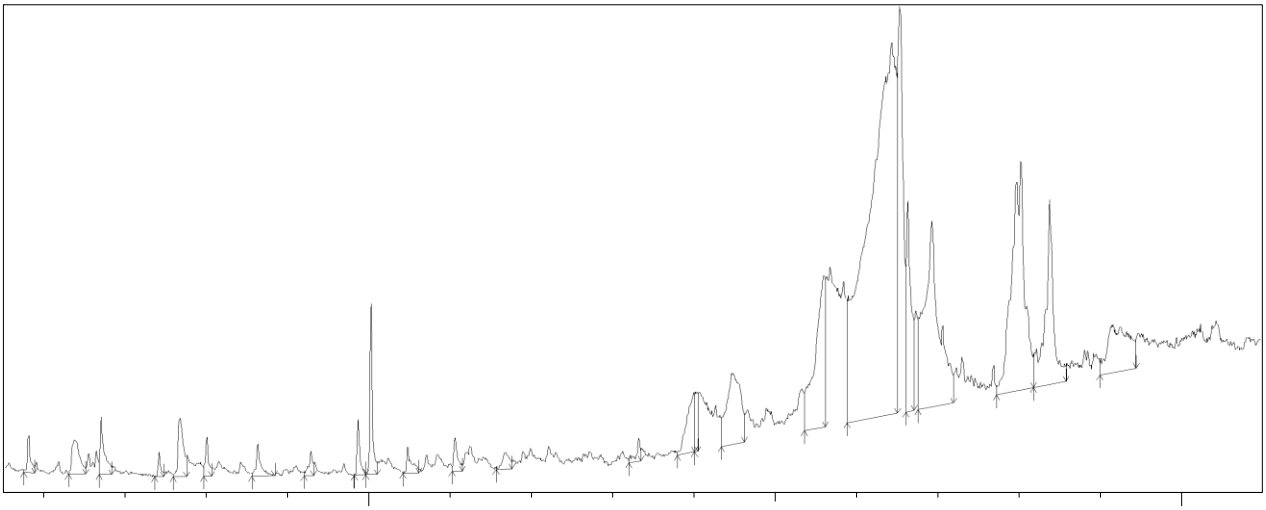


Figure 1: Total Ion Chromatogram (TIC) of the Seed Extract

b) Evaluation of Free Radical Scavenging and Antioxidant Capacity

For the evaluation of the antioxidant capacity, different assays were used to obtain valid results, this is due to the fact that antioxidant compounds present different mechanisms of reactions with the possibility of having synergistic interactions depending on the type of assay used. In this study, the antioxidant potential of the seed extract of *A. cinerea* was evaluated using the DPPH and the galvinoxyl assays (Table 2).

i. *In vitro* DPPH Free Radical Scavenging and Antioxidant Potentials

The results of DPPH radical scavenging assay of seed of *A. cinerea* grown in Nigeria is as shown in Table 2. The seed extract showed concentration-dependent increases in radical scavenging potential. The extract was evaluated at the concentrations of 1000, 500, 250, 125 and 100 μgml^{-1} and with percentage free radical scavenging of 90, 89, 88, 88 and 87%, respectively. The seed exhibited low inhibition concentration (IC_{50}) of 5.0 μgml^{-1} and antioxidant activity index (AAI) of 8.0. The IC_{50} values represent the concentration at which 50% of DPPH is reduced. A low IC_{50} value indicates a potent antioxidant activity. Ascorbic acid showed the inhibition concentration of IC_{50} to be 9 μgml^{-1}. The extract showed a similar antioxidant properties compared to the synthetic antioxidant (ascorbic acid). The seed extract of *A. cinerea* investigated in this study gave more promising free radical scavenging and antioxidant activity than the pulp essential oil of *A. muricata* from Ghana with DPPH IC_{50} of 512 μgml^{-1} (Gyesi *et al*., 2019). The DPPH free radical scavenging and antioxidant of the extract were based on the hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms. The HAT mechanism measures the ability of an antioxidant to quench free radicals by donating hydrogen. HAT-based mechanisms are more relevant to radical chain-breaking antioxidant capacity (Huang *et al*., 2005; Al-Amiery *et al*., 2013; Ololade *et al*., 2014). The SET method measures the ability of antioxidant to transfer one electron to reduce free radical. SET involves two components in the reaction, *i.e.* the antioxidant and oxidant.

SET mechanism measures the abilities of phenolic antioxidants in the extract, to transfer one electron to reduce radicals which changes colour when reduced. The degree of colour change is correlated with the antioxidant potential (Wright et al., 2001; Prior et al., 2005; Ololade *et al*., 2014).

In *vitro* galvinoxyl Free Radical and Antioxidant Potential Galvinoxyl is a stable phenoxy radical that exhibits characteristic UV absorption at 429 nm in methanol solution. The radical have strong absorption in the visible region, while its absorption decreases proportionally upon receiving an electron or hydrogen from the antioxidants. The free radical scavenging potential of the phytochemicals in seed extract was

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obtained based on the absorption change (Lu et al., 2010). The result of galvinoxyl radical scavenging assay of the seed extract of A. *cinerea* grown in Nigeria is shown in Table 2. The extract was evaluated at the concentrations of 1000, 500, 250, 125 and 100 μgml^{-1} and with percentage free radical scavenging of 47, 42, 46, 20 and 19%, respectively. The seed exhibited the low inhibition concentration (IC_{50}) of 100.0 μgml^{-1} and antioxidant activity index (AAI) of 0.4. The seed extract of A. *cinerea* investigated in this study had a lower galvinoxyl free radical scavenging and antioxidant compared to ascorbic acid (the reference compound), which had IC_{50} and AAI values of 15.0 μgml^{-1} and 2.8. The seed extract of A. *cinerea* investigated in this study gave a promising free radical scavenging and antioxidant activity comparable with the rhizome methanolic extract of *Curcuma longa* from Nigeria with galvinoxyl IC_{50} and AAI values of 25 μgml^{-1} and 1.68, respectively (Ololade *et al*., 2020). Generally, the extract investigated showed good antioxidant potential even at very low concentrations. Percentage radical scavenging activity was very low in galvinoxyl assay compared to DPPH assay. The results showed that the steric hindrance among adjacent bulky groups within a galvinoxyl molecule limited the extract to scavenge galvinoxyl radicals effectively unlike DPPH, while extracts showed a powerful capacity for scavenging free radicals in DPPH (Barzegar and Moosavi-Movahedi, 2011; Apak *et al*., 2016; Kubo, 2019; Ololade *et al*., 2020). Natural antioxidants from plants help to maintain an adequate antioxidant status in human body. Antioxidants decrease the oxidative damage directly via reacting with free radicals or indirectly by inhibiting the activity or expression of free radical generating enzymes or enhancing the activity or expression of intracellular antioxidant enzymes (Lu *et al*., 2010; Kurutas, 2016; Ighodaro and Akinloye, 2018).

Table 2: Antioxidant Potential

Extract	IC_{50} μgml^{-1}	AAI
DPPH	5.0	8.0
GALV	100.0	0.4

c) Antibacterial Potential

The antimicrobial potential of the seed extract of *A. cinerea* investigated in this study were tested against eleven clinically isolated multi-drug resistant Gram-negative (seven isolates) and Gram-positive (four isolates) strains of bacteria were investigated using the agar well diffusion method. The extracts investigated in this study demonstrated a broad-spectrum of activities against both Gram-positive and Gram-negative bacteria tested in this study. Table 3 and figure 1 summarize the zones of microbial growth inhibition and antibacterial index by the seed extract of *A. cinerea*, which showed good antibacterial activities against all the clinically isolated organisms. The result of the antimicrobial activity showed that the seed extract have high bactericidal activities from sensitive to ultra-sensitive as compared to cefuroxime (CRX) the synthetic antibiotic used in this study. Based on the value of zone of inhibition, the antibacterial activity potential was dependent on the concentrations of the extract used. Among the tested bacteria, the extract had a zone of inhibition of 30 mm on *P. mirabilis* which indicated that *P. mirabilis* was highly susceptible compared to the other tested bacteria within the concentration of 1000 μgml^{-1} of seed extract of methanol/ethyl acetate of *A. cinerea* in this study. As depicted in Table 3, other high susceptible bacteria at 1000 μgml^{-1} were *Bacillus sp* (25 mm), *P. stuartii* (25 mm), *S. typhimurium* (25 mm), *E. faecalis* (20 mm), *S. marcescens* (20 mm). At the concentration of 500 μgml^{-1} of the seed extract, the bacteria inhibition activities were very high in *S. typhimurium* (25 mm), *Bacillus sp* (22 mm), *E. faecalis* (20 mm), *P. mirabilis* (20 mm), *P. stuartii* (20 mm). The zone of inhibition of the extract at the concentration of 250 μgml^{-1} was significantly different when compared to 1000 and 500 µgml-1 of the extract for the tested bacteria. At a lower concentration of 250 μgml^{-1} of the extract, *E. faecalis* (20 mm) and *P. mirabilis* (20 mm) were more susceptible to the synergic activities of the secondary metabolites in the seed extract, most especially the phenolic compound and the terpenoids. The antibacterial index (AI) ranged between 0.5-25. Comparatively, the antibacterial properties of the extracts investigated in this study have similar antibacterial activities comparable to the leaf essential oil of *A. cherimola* from Egypt which was investigated for its *in vitro* antimicrobial properties against *P. aeruginosa*, *S. aureus*, *B. subtilis*, *B. cereus* with the zones of inhibition of 30, 26, 28 and 35 mm, respectively at 50 µl (Mohammed *et al*., 2016). The differences in the zones of inhibition of the extract could be due to the difference in the levels of their major and minor phytochemical in the seed extract evaluated in this study and the synergetic effect between all the components. The differences in the susceptibility of the tested microorganisms to the extract may also be attributed to a variation in the rate of penetration of the active components of the extract through the cell wall and structures of the cell membrane. With regard to bactericidal effects of natural products, it has been

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frequently postulated that secondary metabolites can penetrate or damage the bacterial cell wall and cell membrane. Once inside the bacterium, the extracts are assumed to trigger the coagulation of cytosolic proteins and the efflux of essential intracellular compounds, and with it the destruction of bacteria. The main advantage of natural antibiotics from plants is that they kill sensitive bacteria by specific mechanisms. One of the ways by which they can kill bacteria consists in the inhibition of peptidoglycan synthesis of the bacteria cell wall. Moreover, they can inhibit bacteria growth via the inhibition of bacteria protein biosynthesis. Other important modes of action are inhibition of DNA topo-isomerase or RNA polymerase inhibition of folic acid synthesis will reduce bacteria enumeration as folic acid is very important for bacteria growth (Apotheken Umschau 2013; Blair *et al*. 2015).

Table 3: Zones of Inhibition (mm) Showing the Antibacterial Properties of the Seed Extract

	ZI of the Seed Extract			Crx
Conc. (μgml^{-1}) / Organisms	1000	500	250	30 μg
Bacillus sp (+)	25	22	19	-
E.faecalis(+)	20	20	20	-
K.pneumoniae(-)	17	17	17	-
M.varians (+)	19	14	14	30
P. aeruginosa(-)	17	13	13	25
P. mirabilis (-)	30	20	20	14
P. stuartii(-)	25	20	15	16
S. agalactiae(+)	19	19	19	-
S. dysenteriae(-)	10	10	10	20
S.marcescens (-)	20	14	-	-
S. typhimurium(-)	25	25	18	-

Key note: Resistant (--), not sensitive (<8 mm), sensitive (9–14 mm), very sensitive (15–19 mm) and ultrasensitive (>20 mm)

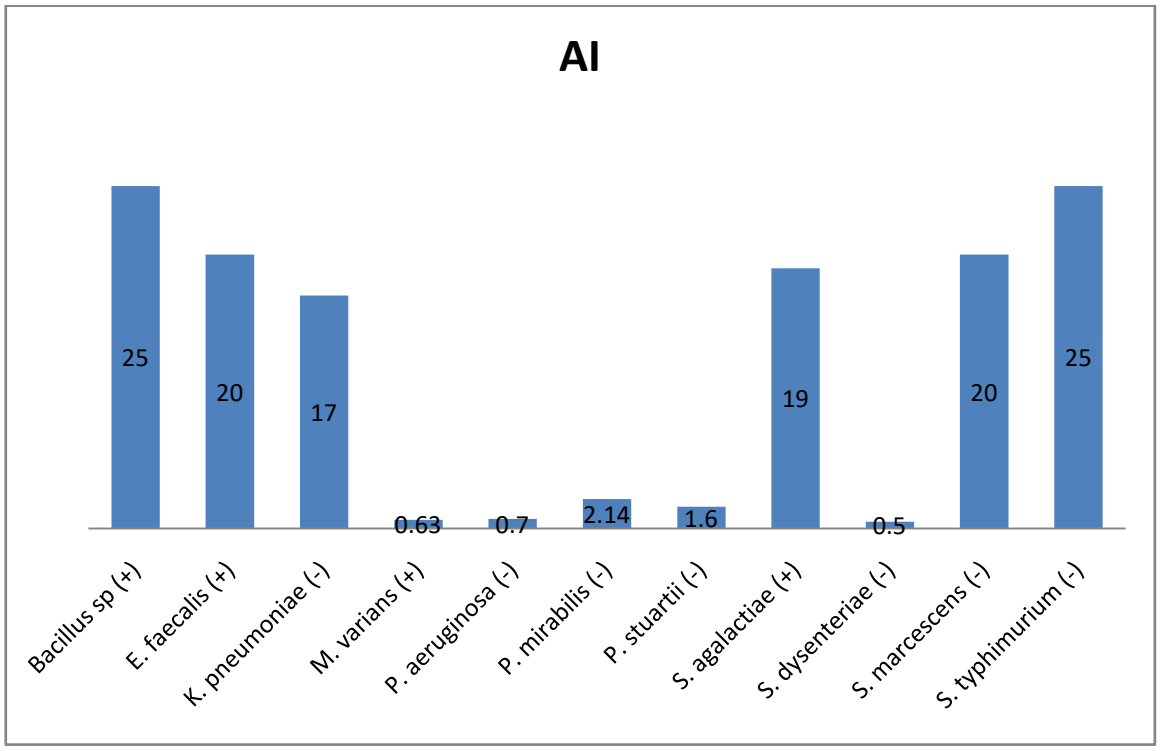


Figure 2: AI of the Extract against the Bacteria Isolates

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IV. Conclusions

This study provides insight into the phytochemical, antioxidant and antimicrobial potential of the seed extract of *A. cinerea*. The study showed that the phytochemicals present in the extract have potential to be used to treat reactive oxygen species (ROS) induced and bacteria health related diseases by inhibiting the free radicals initiating associated with health problems. Further studies into the isolation and identification of phytochemicals that are responsible for the therapeutic potential and their *in vivo* mechanisms of action are necessary for the better understanding of their ability to control diseases that have a significant impact on quality of life. The present finding would be useful for future research directions on the application of the seed from *A. cinerea* in the development of safe drug and preservative for human and animals.

Conflict of Interest Statement: The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of research reported.

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Acknowledgments

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The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

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The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.



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Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

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Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

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Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.



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6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

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11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

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22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

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To make a paper clear: Adhere to recommended page limits.



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- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

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Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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