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## Microbiology and Pathology

Study of  $\alpha$ -Amylase Inhibitory

Tertiary Referral Hospital in Riyadh

Highlights

Fine Needle Aspiration Cytology

Detection of Malignancy in Thyroid Cells

Discovering Thoughts, Inventing Future

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MICROBIOLOGY AND PATHOLOGY

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## CONTENTS OF THE ISSUE

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- i. Copyright Notice
- ii. Editorial Board Members
- iii. Chief Author and Dean
- iv. Contents of the Issue
  
1. Diagnostic Concordance between Fine Needle Aspiration Cytology and Surgical Biopsy of Thyroid Nodules: A Retrospective Cross-Sectional Study from a Tertiary Referral Hospital in Riyadh, Kingdom of Saudi Arabia. **1-5**
2. Automated Cytopathology of Fine Needle Aspiration for the Detection of Malignancy in Thyroid Cells. **7-14**
3. Study of  $\alpha$ -Amylase Inhibitory Activity of Fractions Purified by Chromatographic Methods of the Endophytic Fungus *Penicillium Brevicaule Alba-CC200*. **15-20**
  
- v. Fellows
- vi. Auxiliary Memberships
- vii. Preferred Author Guidelines
- viii. Index





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# Diagnostic Concordance between Fine Needle Aspiration Cytology and Surgical Biopsy of Thyroid Nodules: A Retrospective Cross-Sectional Study from a Tertiary Referral Hospital in Riyadh, Kingdom of Saudi Arabia

By Mohammed Alswayyed

*King Saud University*

**Abstract- Objective:** To assess the diagnostic performance and yield of fine needle aspiration cytology (FNAC) of thyroid nodules.

**Design:** Retrospective study.

**Setting:** Archives of the Pathology Department at King Saud University Medical City, Saudi Arabia between January 2016 and May 2021.

**Subjects:** Overall, 3867 FNAC results from 2442 patients with thyroid nodules with available histopathological follow-up material were included in this study.

**Intervention:** Comparison of the FNAC diagnostic performance with the final histopathological diagnoses.

**Keywords:** *fine needle aspiration cytology, histopathology, sensitivity, negative predictive value, positive predictive value.*

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**Intervention:** Comparison of the FNAC diagnostic performance with the final histopathological diagnoses.

**Main outcome measure:** Evaluation of the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of FNAC.

**Results:** Out of the 3867 FNAC results, 518 (13.4%) cases had histopathology results. Analysis of FNAC results revealed that 278 (53.7%) as benign, 98 (18.9%) as malignant, and 90 (17.4%) as indeterminate lesions, whereas 29 (5.6%) were suspicious for malignancy and 23 (4.4%) were non-diagnostic/unsatisfactory. The rate of false negatives was 30% and 18.5% for indeterminate cases and overall FNAC performance, respectively. Concordance between FNAC and histopathology results was observed in 415 (80.1%) cases. The overall sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of FNAC were 60.0%, 97.5%, 95.3%, 73.8%, and 80.0%, respectively.

**Conclusion:** FNAC was confirmed to be a useful screening procedure for the assessment of patients with thyroid nodules because it is minimally invasive and cost-effective. However, there is a need to address false positive and false negative errors, particularly among indeterminate cases and cases suspicious for malignancy, through careful specimen collection and interpretation of FNAC results in order to improve the diagnostic yield.

**Keywords:** fine needle aspiration cytology, histopathology, sensitivity, negative predictive value, positive predictive value.

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## I. INTRODUCTION

Fine needle aspiration cytology (FNAC) is a minimally invasive procedure commonly used as a diagnostic tool for the evaluation of single thyroid nodules. It is a simple procedure that is safe, cost-effective, and has a high diagnostic accuracy.<sup>[1]</sup> The reported overall sensitivity of FNAC for thyroid cancer ranged from 61.5% to 72%, while specificity was around 98% to 99%.<sup>[2]</sup> For uncommon thyroid malignancies, including anaplastic thyroid carcinoma and metastatic carcinoma, the reported sensitivity of FNAC reached almost 100% and specificity was over 80%.<sup>[3]</sup> As for solitary thyroid nodules, the reported sensitivity and specificity of FNAC were 80% and 86.6%, respectively.<sup>[4]</sup> However, several limitations of FNAC have been reported, including its high rates of unsatisfactory sampling, inconclusive reports, and indeterminate interpretations.<sup>[1]</sup> For papillary thyroid carcinomas (PTC), the sensitivity of FNAC depends on the tumor size. APTC <0.5 cm or >3 cm in size is more difficult to successfully aspirate as compared to tumors that are 0.5 cm to 3 cm in size.<sup>[5]</sup>

False negative thyroid aspirates are identified in 2.7% to 4% of cases diagnosed via FNAC, usually due to interpretation errors in cases of poorly-developed features of malignancy, scant cellularity in the specimen, and suboptimal material.<sup>[6, 7]</sup> False positives occur in up to 8% of cases due to the over diagnosis of follicular neoplasms.<sup>[7]</sup> False negative diagnoses occur in follicular cases, cystic PTCs, and papillary microcarcinomas.<sup>[8]</sup> It was previously suggested that the appropriate technique and preparation can increase the sensitivity and specificity of FNAC.<sup>[9]</sup>

Over the past decades, FNAC has been a primary diagnostic and screening tool for thyroid nodules. It has also been used for the cytologic diagnosis of thyroid cancers that require surgery. Therefore, to avoid unnecessary surgery, this method must yield correct and appropriate diagnoses through proper sampling and interpretation of results. The

present study aimed to evaluate the diagnostic performance of FNAC for thyroid nodules.

## II. SUBJECTS AND METHODS

A retrospective review of medical records of patients who underwent FNAC and histopathological examination of thyroid nodules at the Pathology Department of King Saud University Medical City from January 2016 to May 2021 was conducted. Patients with thyroid FNAC results and available histopathological follow-up results were included in the study. Those without histopathological follow-up results and with a final histopathological diagnosis of microcarcinoma were excluded from the analysis. Overall, 3867 FNAC and surgical biopsy results were reviewed, which comprised 1693 (43.8%) FNAC examinations and 2174 (56.2%) histopathological examinations from a total of 2442 patients. Of the 3867 results, 592 (15.3%) patients had both FNAC and histopathology results. After excluding histopathological biopsies that revealed microcarcinoma, we compared the FNAC results and histopathological examinations of 518 patients. Information gathered included the age and sex of the patients, the location of the FNAC specimen collection, and the FNAC and histopathological results.

The findings of thyroid FNAC and histopathology were compared. The characteristics and clinical relevance of FNAC were tested by calculating the values of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) which are all expressed as percentages.

The data were analyzed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA).

This study was approved by the relevant Institutional Review Board. The need for informed patient consent was waived due to the retrospective nature of this study. None of the patients could be identified in the course of the study or during the publication and analysis of results.

## III. RESULTS

Overall, 518 patients were included which consisted of 420 (81.5%) women and 96 (18.5%) men. Location of the nodules differed among the patients, with 193 (37.3%) of the nodules involving the right thyroid gland, 168 (32.4%) involving the left thyroid gland, 142 (27.4%) involving both the left and right thyroid glands, and 15 (2.9%) had nodules involving the midline or isthmus (Fig 1).

Results of FNAC indicated that 278 (53.7%) are benign lesions, 98 (18.9%) malignant lesions, 90 (17.4%) indeterminate lesions, 29 (5.6%) lesions suspicious for malignancy and 23 (4.4%) lesions that were non-diagnostic/unsatisfactory. The most common type of benign lesion identified using FNAC was follicular nodule which was identified in 238 (85.6%) patients. PTC was the most common malignancy (86.7%) identified using FNAC (Table 1). Through histopathology, 271 (52.3%) benign lesions and 247 (47.7%) malignant lesions were identified. The most common benign lesions detected using histopathology were follicular nodules in 221 (81.5%) patients, whereas papillary carcinoma was the most common malignant diagnosis based on histopathology (n=200, 81.0%)(Table 2).

Table 1: FNAC findings in thyroid specimens (n = 518)

Diagnosis on FNAC	n (%)
Benign	278 (53.7%)
Follicular nodules	238 (85.6%)
Hashimoto's thyroiditis	31 (11.2%)
Hurthle cell adenoma	9 (3.2%)
Malignant	98 (18.9%)
Papillary carcinoma	85 (86.7%)
Follicular carcinoma	2 (2.0%)
Medullary carcinoma	3 (3.1%)
Anaplastic large cell carcinoma	3 (3.1%)
Hurthle cell carcinoma	5 (5.1%)
Indeterminate	90 (17.4%)
Suspicious for malignancy	29 (5.6%)
Unsatisfactory/non-diagnostic	23 (4.4%)

Abbreviations: FNAC, fine needle aspiration cytology

**Table 2:** Histopathological diagnosis in thyroid specimens (n = 518)

Histopathological diagnosis	n	%
Benign	271	52.3%
Multinodular goiter	221	81.5%
Hashimoto's thyroiditis	41	15.1%
Hurthle cell adenoma	9	3.3%
Malignant	247	49.1%
Papillary CA	200	81.0%
Follicular CA	30	12.2%
Medullary CA	6	2.4%
Anaplastic large cell CA	4	1.6%
Hurthle cell CA	7	2.8%

Abbreviations: CA, carcinoma

Of the 98 malignant lesions identified through FNAC, eight (8.2%) were considered benign through histopathological examination. Out of the 278 benign lesions identified through FNAC, 82 (29.5%) were considered malignant through histopathological examination. Four (17.4%) lesions considered unsatisfactory through FNAC examination were

classified as malignant through histopathology. In three (10.3%) FNAC cases reported as suspicious for malignancy, FNAC revealed a benign lesion. A malignant lesion was found through histopathology in half of the lesions considered indeterminate by FNAC (Table 3).

**Table 3:** FNAC diagnosis versus final pathological diagnosis of specimens (n = 518)

FNAC diagnosis	Total (%)	Final pathological diagnosis (n=518)	
		Malignant	Benign
Positive for malignancy	98 (18.9%)	90 (91.8%)	8 (8.2%)
Negative for malignancy	278 (53.7%)	82 (29.5%)	196 (70.5%)
Unsatisfactory/Non-diagnostic	23 (4.4%)	4 (17.4%)	15 (82.6%)
Indeterminate	90 (17.4%)	45 (50.0%)	45 (50.0%)
Suspicious for malignancy	29 (5.6%)	26 (89.7%)	3 (10.3%)
Total	518	247 (47.7%)	271 (52.3%)

Abbreviations: FNAC, fine needle aspiration cytology

FNAC showed the following sensitivities, specificities, PPVs, and NPVs, respectively: 100%, 40.0%, 94.7%, and 100% for positive cases; 20.0%, 100%, 100%, and 75.7% for negative cases; 25.0%, 100%, 50.0%, and 81.8% for unsatisfactory cases; 40.0%, 100%, 100%, and 62.8% for indeterminate cases; and 100%, 33.3%, 92.9%, and 100% for lesions suspicious for malignancy. The rate of false negative results in indeterminate cases was 30.0%, whilst that for FNAC was 18.5%. Concordance between FNAC and histopathology results was observed in 415 (80.1%) cases. Overall, FNAC exhibited sensitivity, specificity, PPV, NPV, and accuracy of 60.0%, 97.5%, 95.3%, 73.8%, and 80.0%, respectively.

#### IV. DISCUSSION

Several studies on the diagnostic sensitivity and reliability of FNAC for detecting the presence or absence of thyroid diseases have reported an FNAC sensitivity ranging from 43% to 99% and thyroid FNAC specificity ranging from 72% to 100%.<sup>[7, 8, 10]</sup>

In the current study, we found a 19.9% discordance between FNAC and histopathology results, which generally varies between 30% to 40%, depending on the rate of false positives and false negatives.<sup>[11]</sup> The most commonly reported causes of discordance

between FNAC and histopathology include interpretation errors (false positives), sampling errors (false negatives), and unsatisfactory samples.<sup>[12]</sup> Discordance often occurs among cases with overlapping cytological features, particularly among adenomatous nodules, follicular neoplasms, follicular variants of PTC, and Hashimoto's thyroiditis.<sup>[12]</sup>

In this study, we had 96 (16.5%) false negatives, most of which were misdiagnosed as negative for malignant lesions or were due to non-diagnostic FNAC sampling and indeterminate sample issues. Many of the FNAC-diagnosed as false negative follicular lesions were found to be malignant PTCs. The high false negative rates in cases with large thyroid nodules can be explained by the difficulty in precisely sampling the entire nodule.<sup>[13, 14]</sup> Nodule heterogeneity has been suggested as one of the reasons why it is difficult to evaluate certain thyroid nodules by FNAC.<sup>[15]</sup> Sampling errors occur when the targeted nodule is missed or hidden in cases of a multinodular thyroid.

Seven of the thyroid FNACs with histopathologically proven false positive diagnosis included focal nuclear atypia and Hashimoto's thyroiditis, which were misdiagnosed as PTC. Focal nuclear atypia is often mistaken for PTC due to the overlapping features including papillary hyperplasia and

chromatin clearing, which are very common in hyperplastic nodules.<sup>[16, 17]</sup> Hashimoto's thyroiditis can be mistakenly diagnosed as PTC due to the atypical nuclear features, including nuclear grooves, overlapping nuclei, and nuclear clearing.<sup>[18]</sup> One way to distinguish Hashimoto's thyroiditis from papillary microcarcinomas is when solid cell nests are numerous and also by immunohistochemistry, particularly of p63, thyroid nuclear factor-1, thyroglobulin, Hector Battifora mesothelial-1, and calcitonin.<sup>[18, 19]</sup>

Overall, our study revealed an FNAC sensitivity, specificity, PPV, NPV, and accuracy of 60.0%, 97.5%, 95.3%, 73.8%, and 80.0%, respectively. The sensitivity and accuracy we observed are relatively lower compared to results of recent studies with similar methodologies showing a sensitivity of 80% and an accuracy over 90%.<sup>[20-22]</sup> The lower sensitivity of FNAC in this study was due to a large number of false negatives in the indeterminate cases and in those with follicular neoplasm with papillary-like nuclear features. Indeterminate cases arise when pathologists are challenged in defining the FNAC results as clearly benign or clearly malignant. This is due to questionable features, such as the presence or absence of vascular capsular invasion, papillary-like nuclear features, and atypia of undetermined significance.<sup>[23]</sup> Several procedures have been found to improve the detection of malignancy in indeterminate smears, including real-time polymerase chain reaction of molecular markers that have been shown to have high sensitivity and specificity,<sup>[24,25]</sup> as well as elastography.<sup>[26, 27]</sup>

Recently, additional measures have been suggested to reduce the diagnostic error in thyroid FNAC and histopathology.<sup>[11]</sup> These include the use of uniform terminology for reporting cases, together with a review of the clinical and ultrasound findings and a multidisciplinary approach.<sup>[11]</sup> Furthermore, proper and strict specimen collection, implementation of specimen adequacy rules, and awareness of certain FNAC pitfalls may also improve the interpretation of FNAC and diagnosis of thyroid nodules.<sup>[7, 8]</sup>

We recognize that our study has some limitations. Selection bias may result from the retrospective nature of the study along with the study being conducted in a single center. However, we were able to draw and highlight some important findings on the reliability of FNAC in the assessment of thyroid nodules.

## V. CONCLUSION

Our study confirmed the usefulness of FNAC as a screening procedure for the assessment of patients with thyroid nodules. However, this study showed low sensitivity and NPV of FNAC because of the relatively high number of false negatives. Nevertheless, it is crucial, particularly among indeterminate cases and

those suspicious of malignancy, to exercise meticulous specimen collection and careful interpretation of FNAC results to improve diagnosis.

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*Fig. 1:* Sources of thyroid specimens (thyroid gland localization) for 518 FNAC and histopathological examinations



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# Automated Cytopathology of Fine Needle Aspiration for the Detection of Malignancy in Thyroid Cells

By Gabrielly Pereira Ribeiro, Carlos Musso & Dominik Lenz

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**Abstract-** Cytopathology of thyroid cells is an established method to detect malignancies in the thyroid. It is of advantage because an anesthesia and a diagnostic laparotomy is not necessary. There are however not yet many studies about automated cytopathology in thyroid cells. To this end, the aim of the present study was to establish an automated diagnosis of malignancy using image analysis and subsequent machine learning and Artificial intelligence. Light microscopy images of 52 patients were analyzed and the results were compared to those of pathology. The results of the automated analysis yielded a sensitivity of 0.94 and a specificity of 0.91 when compared to those of the pathologic diagnoses. The process of machine learning yielded an under curve area of 0.91 as calculated by a ROC-curve. The software used for image analysis, machine learning and classification (diagnosis) are open-source software, respectively.

**Keywords:** *machine learning; cellprofiler; image cytometry; cytopathology; papillary thyroid carcinoma.*

**GJMR-C Classification:** *LCC: RC 280 .T5*



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Gabrielly Pereira Ribeiro <sup>α</sup>, Carlos Musso <sup>σ</sup> & Dominik Lenz <sup>ρ</sup>

**Abstract-** Cytopathology of thyroid cells is an established method to detect malignancies in the thyroid. It is of advantage because an anesthesia and a diagnostic laparotomy is not necessary. There are however not yet many studies about automated cytopathology in thyroid cells. To this end, the aim of the present study was to establish an automated diagnosis of malignancy using image analysis and subsequent machine learning and Artificial intelligence. Light microscopy images of 52 patients were analyzed and the results were compared to those of pathology. The results of the automated analysis yielded a sensitivity of 0.94 and a specificity of 0.91 when compared to those of the pathologic diagnoses. The process of machine learning yielded an under curve area of 0.91 as calculated by a ROC-curve. The software used for image analysis, machine learning and classification (diagnosis) are open-source software, respectively. It may be concluded that the method presented in the present study may be used for a low-cost verification of pathological diagnosis. The equipment used for this study (light microscopy, digital camera and a Personal Computer) is available in almost every laboratory.

**Keywords:** machine learning; cellprofiler; image cytometry; cytopathology; papillary thyroid carcinoma.

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Brazil.

## I. INTRODUCTION

**H**istopathology (HP), a technique that aims to diagnose diseases in tissue sections using microscopy, established in 1893, remains the gold standard until nowadays (Tiford et al., 2006; den Baaker, 2017). In 1928, Papanicolaou described a new technique of cell-based diagnosis called Cytopathology (CP) (Van den Tweel and Taylor, 2010).

Results, advantages and disadvantages of HP and CP have always been subject of comparison (Crino et al., 2021; Gerstner, Tarnok, 2002; de Ruyter et al., 1994). Recent literature reveals that the results of HP and CP in terms of accuracy became comparable, independent if CP is conducted manually (de Ruyter et

al., 1994) or automatically (Gerstner et al., 2002; Gerstner et al., 2009).

The studies of Gerstner et al. used Laser Scanning Cytometry (LSC) aiming to define the DNA-ploidy as a marker of malignancy. LSC is automated and thus reproducible, but at least the equipment is cost-intensive. A manual count is low cost but tedious, time-consuming and its results are not reproducible. To this end the authors of the present study aimed to combine an automated analysis that is less expensive. The authors hypothesized that the morphological features yield information about the malignancy/benignancy of the respective sample.

The aim of the present study was to analyze light microscopic images of cytopathologic samples of thyroid cells using free software. The classification (diagnosis) was aimed to be conducted by artificial intelligence after a process of machine learning.

Samples of thyroid were chosen because the incidence of diagnosed thyroid cancers is increasing at least in Brazil and the USA. At least in those two countries, thyroid cancer is the fifth most frequent cancer in women. As stated in the recent scientific literature, an inexpensive but reliable methodology will always be an important issue to lower the costs for reliable medical diagnosis (Gerstner et al., 2002; Mittag et al., 2006; Gerstner et al., 2009; Buzin et al., 2015; Laffers et al., 2006; Melzer et al., 2016).

## II. MATERIALS AND METHODS

### a) Study Design

Manual diagnostic data from cytopathological aspirates were provided by Cassiano Antonio Moraes University Hospital. The automated analysis was conducted at the Image Cytometry Laboratory 10 (B) at the Biopractices Campus of Vila Velha University. The type of study was cross-sectional where the observation of data will occur within a time interval, without the need for direct interaction with the patients.

### b) Obtaining of Samples and Images

In the present study 52 samples submitted to fine needle aspiration stained by pap smear were collected with a diagnosis made by the pathologist Dr. Carlos Musso; selected from March/2021 to March/2022. The photos taken by the pathologist

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himself were collected and sent to the researcher. The study was previously approved by the ethics committee of the institution Vila Velha University - (No. 4.094.607) and of the institution Cassiano Antonio Moraes University Hospital - (No. 5.073.110).

c) *Image Preparation and Analysis*

The images have been saved in .tif format. Samples of each patient were stored in individual folders without personal identification. The received images were analyzed and classified using free software. The

software chosen for nuclei analysis was CellProfiler (CP) (version 4.2.4), after the pipeline (Figure 1) was created in this software, the images of each patient were loaded, there was a background inversion where the colors were inverted to grayscale scales for processing the pixels, presenting you light objects on a dark background, identification of all objects (nuclei) and finally filtered (Figure 2 and 3), in order to exclude non-cellular artifacts.

Pipeline Used with Cellprofiler for All Samples

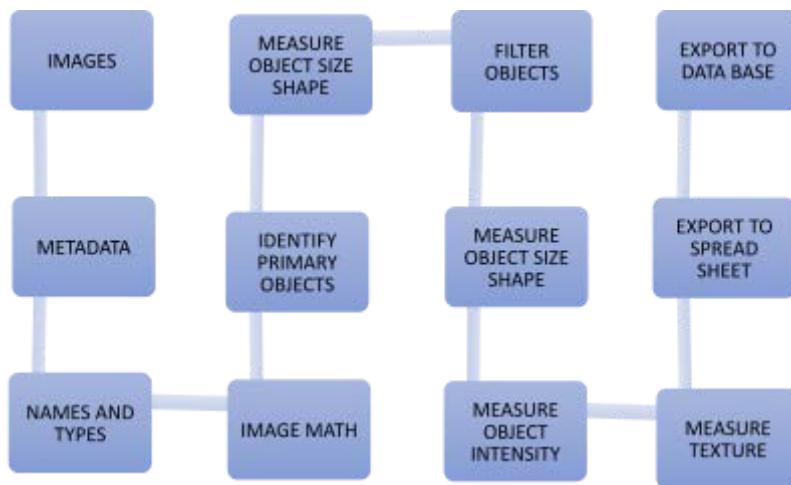


Figure 1: Pipeline: First all the images were loaded, identified by the unique format known in all .tif, the intensities were inverted for the objects of interest asking for light on a dark background, later the primary object was identified as nuclei 1, after filtering the objects (eliminating objects unexpected) a new count of the parameters was performed and then data nuclei 2 was found, only these were exported to carry out the classification.

Steps Using Cellprofiler – Malignant Sample

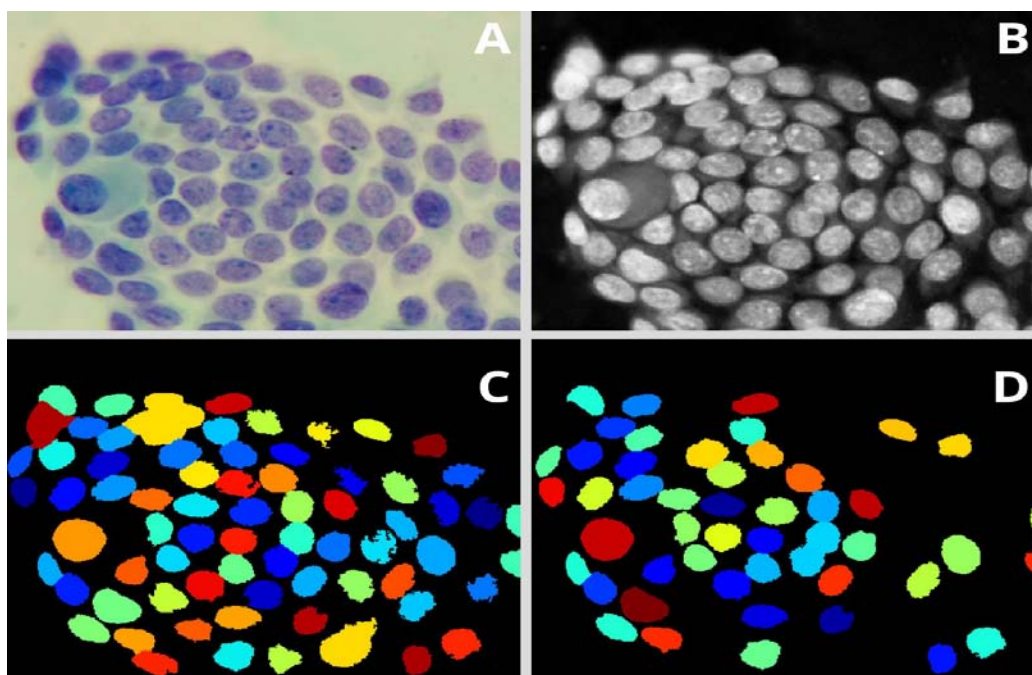


Figure 2: Analysis performed in Cellprofiler of a malignant sample. A: Original Image, B: Color inversion to grayscale, C: Nuclei identification D: Filter applied to exclude the artifacts.

Steps Using Cellprofiler – Benignant Sample

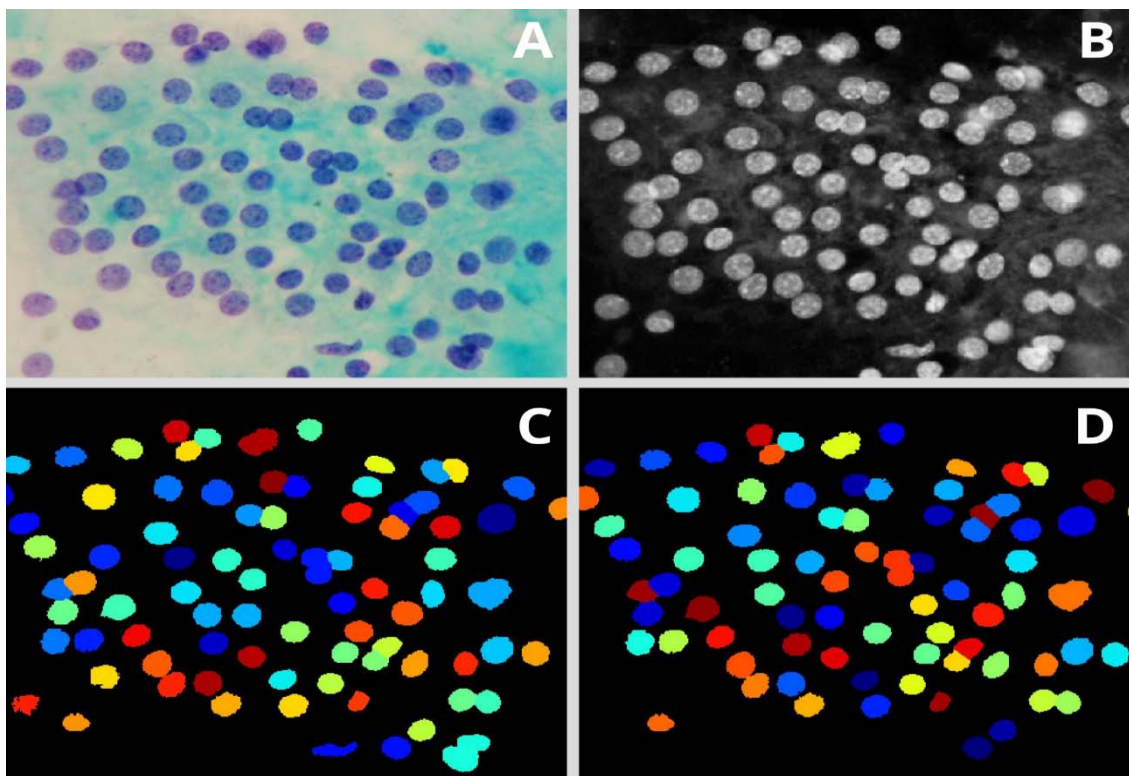


Figure 3: Analysis performed in Cellprofiler of a benignant sample. A: Original Image, B: Color inversion to grayscale, C: Nuclei identification D: Filter applied to exclude the artifacts.

The dates of the analyzed nuclei were exported to an excel file and later to another two software that performed the classification by supervised learning (Cellprofiler Analyst (CPA) and Tanagra (TG) software).

d) Statistical Analysis

After the complete analysis of all samples a comparison of the results of the effectiveness of the automated method and the manual diagnosis was made with MedCalc software (<https://www.medcalc.org/>

download/). Also, calculations for specificity and sensitivity with CPA and TG software.

III. RESULTS

The ROC curve (Figure 4) shows the progress of machine learning (AUC = 0.96), and the capability to differentiate the positive and negative.

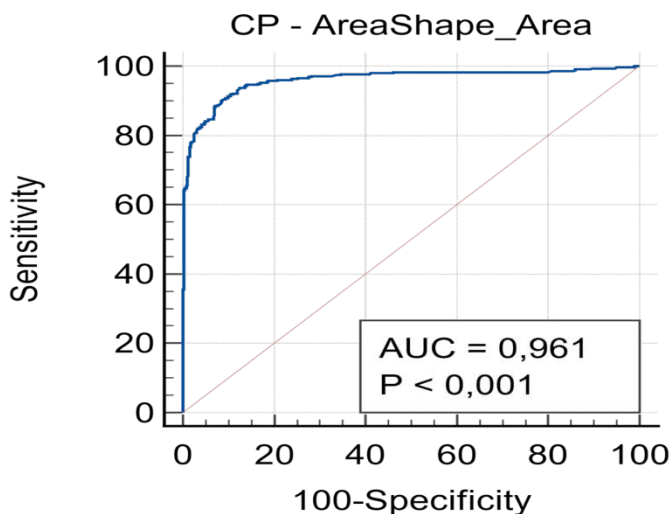


Figure 4: ROC curve between automated and manual results.

TG is a macro to be integrated in excel for classification purposes, amongst others. If, like in the present study there are multiparametric data, TG offers the possibility to choose the best parameters for a

successful classification (data mining), that is, parameters that do not substantially enhance the results will be excluded in this step.

The scatterplot shown in figure 5 concluded that the software was able to correctly differentiate the classes.

Scatterplot with Software Tanagra – Supervised Learning

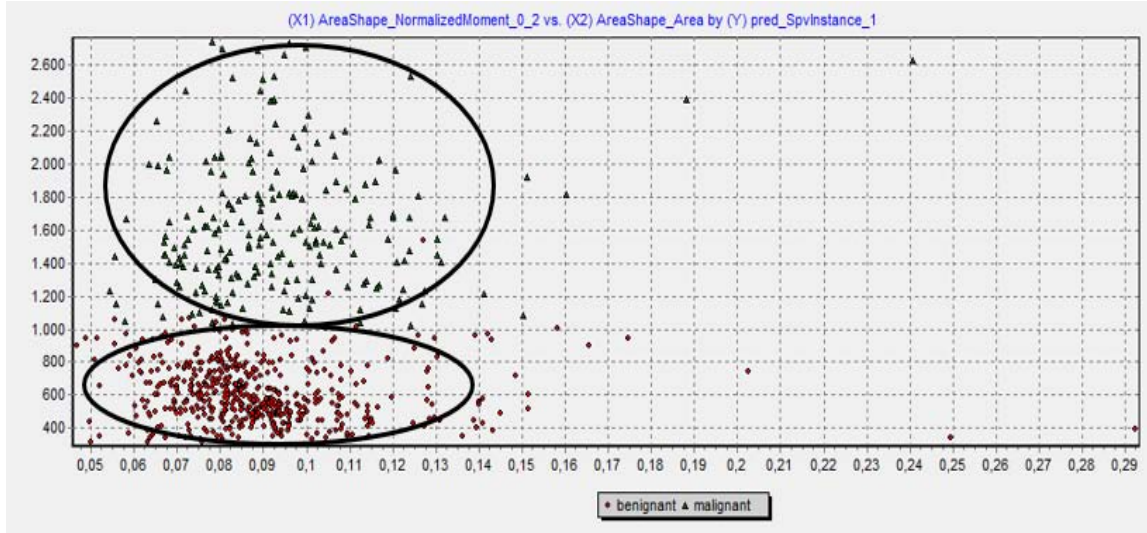


Figure 5: Classification obtained in the TANAGRA software (Scatter plot: red-benign / green-malignant).

The algorithm chosen by the authors was the Linear discriminant analysis (LDA). LDA is strongly related to analysis of variance (ANOVA) and regression analysis, which also attempt to express a dependent

variable as a linear combination of other characteristics (Venkataraman et al., 2007). TG software presented data with high sensitivity (0.94) and specificity (0.91) (Figure 6).

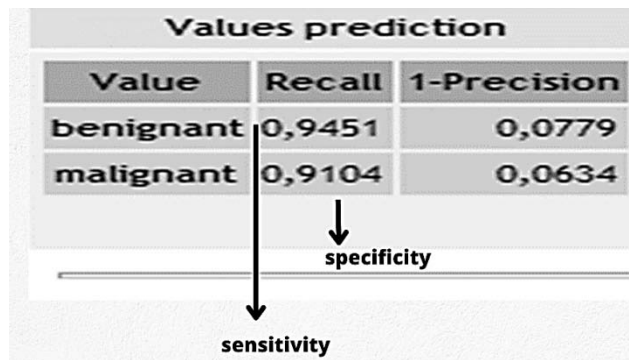


Figure 6: Linear discriminant analysis between benignant and malignant objects.

The other software used to also classify the data was CPA, which also received the same data calculated by TG software. Figure 7 represents the training manual classification made. CPA also supervised machine learning and the algorithm used was logistic regression. In this software the results were comparable to the TG software and also showed high performance in learning – 97.08% of accuracy.

### Cellprofiler Analyst Training

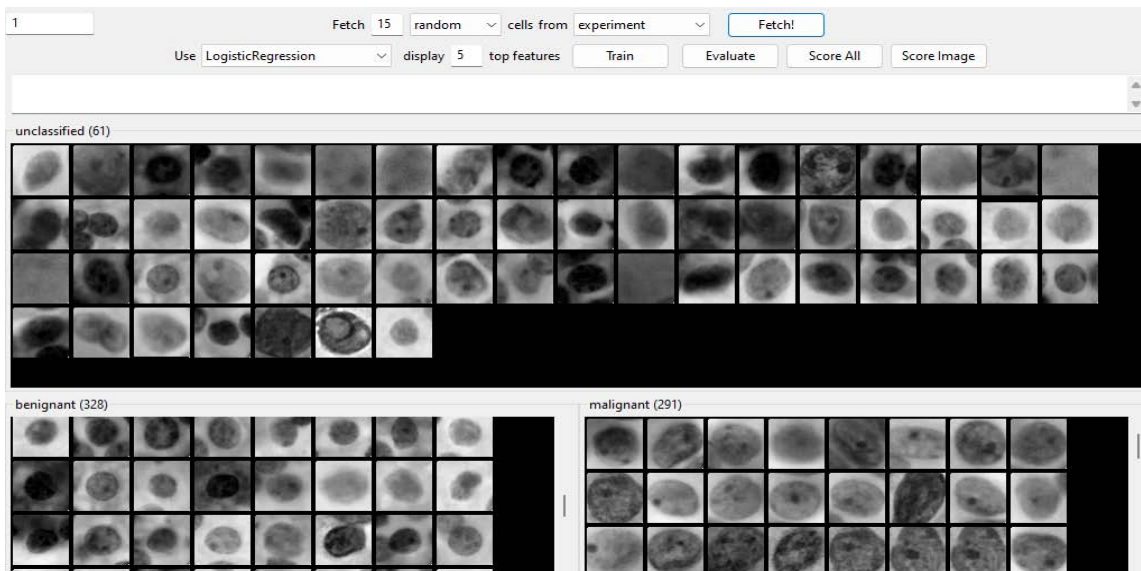


Figure 7: Training with two classes, malignant (lower right) and benign (lower left) cells.

After training benignant and malignant objects were requested to verify software performance to calculate sensitivity and specificity. (Figure 8 and 9).

### Cellprofiler Analyst Performance - Benignant

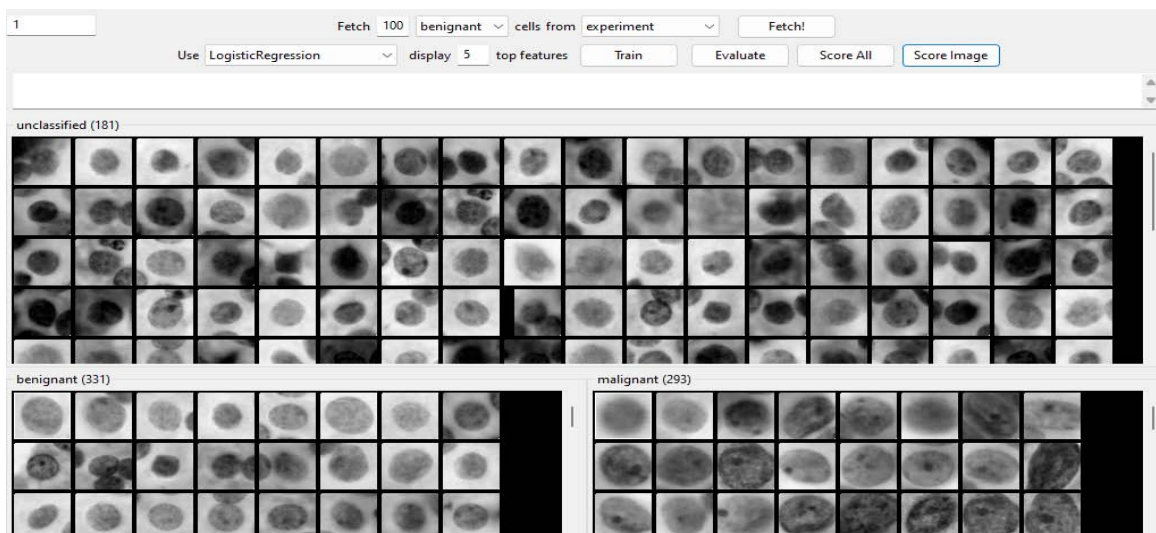


Figure 8: 100 benignant correct objects requested after training.



Cellprofiler Analyst Performance - Malignant

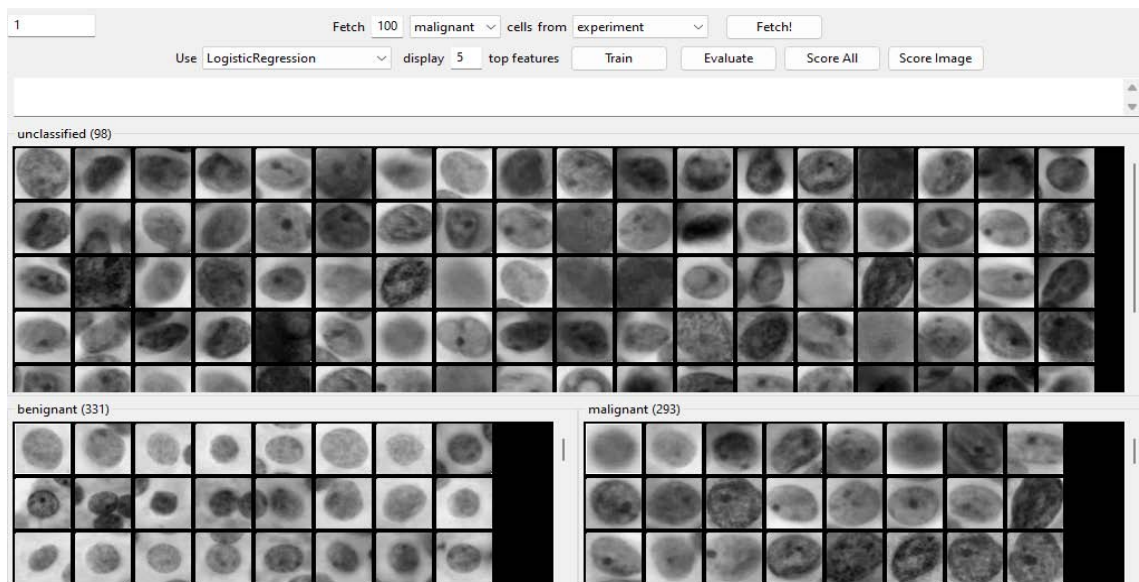


Figure 9: 100 malignant correct objects requested after training.

To check the machine learning progress, a score image tool is displayed after training to show the correctness of the machine (Figure 10).

Score Image of Malignant and Benignant Nucleus – Cellprofiler Analyst

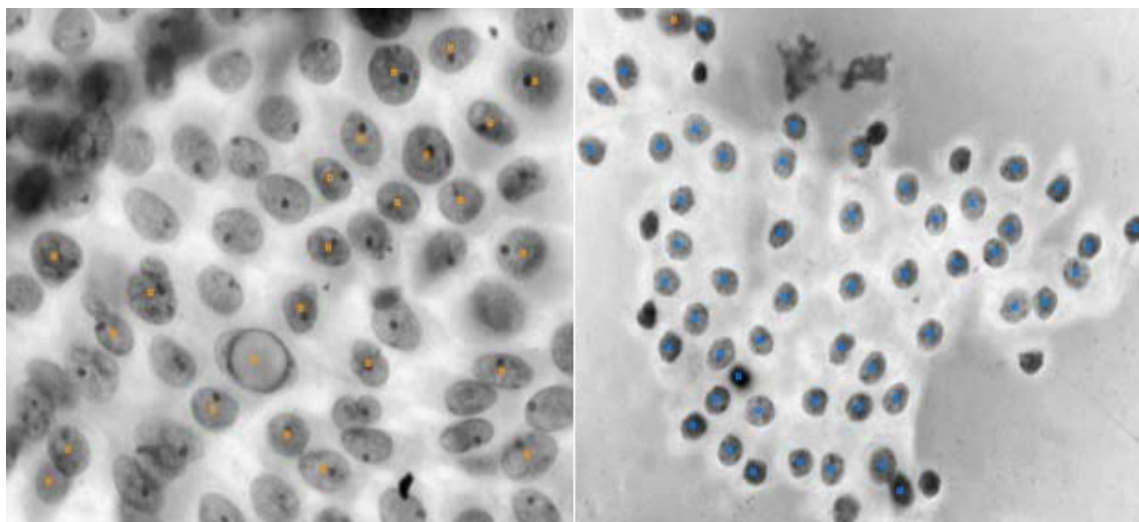


Figure 10: Score Image: This tool allows the analyst to see the full extent of the image and the objects there identified according to the classification performed under each class after training. A: Malignant objects identified B: Benignant objects identified. - 97.08% of accuracy.

The results calculated by CPA and TG obtained accuracy above 90%. Both software were able to distinguish the difference between classes. Regardless of the number of samples, the algorithm is able to learn and perform the correct classification of objects, as shown in the results of the present study.

IV. DISCUSSION

Fine-needle aspiration cytology is a routinely used, reliable, quick, highly reproducible, minimally invasive, and cost-effective method for diagnosing

nodular lesions of the thyroid (Rosai, 1996). It has been suggested as a first step test in the diagnosis of thyroid nodules (Segev et al., 2003; Aguilar et al., 1997). The most important contribution of this method to the diagnostic process is its power to distinguish patients who need surgical intervention from those who do not (Koss, 1992). Manual diagnosis, however, is time-consuming and not reproducible.

Currently there are free software where it is possible to view all objects and classify them with the best parameters. After training all objects, the software

can even independently predict a correct diagnosis in new objects. Other studies also had satisfying results with data found in automated analysis of cellular objects (Deka et al., 2017).

An interesting study combining machine learning and papillary thyroid carcinoma published in 2021 showed several machine learning models were built to predict disease recurrence and all models had a confirmed accuracy of 90% or more (Park & Lee, 2021).

Automated quantitative analysis of cellular objects is currently considered the viable option in developed countries, because the scientific community proves that human-machine interaction has the potential to reduce human effort and increase confidence in algorithmic decision to solve clinical diagnoses (Jayatilake & Ganegoda, 2021; Sarker, 2021).

In the literature, there are few studies with images of the cell classification process (Wang et al., 2010; Wang et al., 2019), unlike this study, which the objective was to show step by step.

In terms of DNA ploidy there may be a low percentage of false positive artifacts. However, those may not have influenced the results of the present study as proven by the high accuracy of the results (Gerstner et al., 2006). The ROC curve was created only using the area of the nuclei, concluding the area of the nuclei is referring to the amount of DNA, which makes an analysis of the ploidy relatively easy, even when using light microscopy as in the present study.

The term digital pathology is considered as the future of histopathology. The authors agree that the integration of images and digital results is not yet standard, but it will significantly contribute to receiving digital pathology as part of the clinical evaluation (Athellogou & Huss, 2018; Ribeiro et al., 2019). Other studies concluded that after several highly accurate and scientifically proven studies, histopathology is ready for automation (Ribeiro et al., 2020; Lucas et al., 2021).

The main purpose of this study was to compare the diagnosis manual analysis (gold standard) and automated analysis. The results are comparable to the gold standard with high specificity and high sensitivity in two different classifiers.

*Conflicts of Interest*  
Nothing to declare.

## ACKNOWLEDGMENTS

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## Study of $\alpha$ -Amylase Inhibitory Activity of Fractions Purified by Chromatographic Methods of the Endophytic Fungus *Penicillium Breviceale Alba-CC200*

By Mukhammedov Iqbol, Gulyamova Tashkhan, Ruzieva Dilaram,  
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**Abstract-** In this study, the antidiabetic activity of purified fractions of the endophytic fungus *Penicillium breviceale alba-CC200* isolated from the plant *Celosia cristata* was investigated. Antidiabetic activity was evaluated by inhibition of  $\alpha$ -amylase enzyme. The secondary metabolites of *P. breviceale alba-CC200* were partially purified by column chromatography and obtained fraction II inhibited  $\alpha$ -amylase by 91.2%. High inhibitory activity was observed in fraction II and was determined to be cardiac glycosides and terpenoids by thin layer chromatography (TLC) and qualitative analysis. This requires a more precise study of the purified fractions using high-precision chromatographic methods.

**Keywords:** *a-amylase, a-glucosidase, column chromatography, TLC, cardiac glycosides, terpenoids.*

**GJMR-C Classification:** DDC Code: 579.173 LCC Code: QR351



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# Study of $\alpha$ -Amylase Inhibitory Activity of Fractions Purified by Chromatographic Methods of the Endophytic Fungus *Penicillium Brevicaule Alba-CC200*

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& Turdimuhammadova Sarvinoz <sup>¥</sup>

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**Keywords:** *a-amylase, a-glucosidase, column chromatography, TLC, cardiac glycosides, terpenoids.*

## I. INTRODUCTION

According to the World Health Organization, there are more than 400 million diabetic patients in the world today. According to analysis, this figure may exceed 550 million by 2030. In Uzbekistan, 220,000 people have been registered with this disease [1]. In recent years, diabetes has taken the third place among the causes of death, and cardiovascular and oncological diseases are in the second place. The social consequences of diabetes are also negative, because the treatment of this disease requires huge costs from the health care system [2]. Diabetes mellitus is a disease characterized by the presence of chronic hyperglycemia accompanied by metabolic disorders of carbohydrates, lipids and proteins [3].

In the last decade, a number of new methods of diabetes treatment have been proposed, but three areas remain the main ones: a) diet, insulin for patients with type 1 diabetes, b) hypoglycemic drugs used for type 2 diabetes, c) general procedure and physical activity [2].

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There are many different therapeutic approaches in the treatment of type 2 diabetes. Inhibition of carbohydrate-hydrolyzing enzymes such as  $\alpha$ -amylase is one of the important approaches to lowering postprandial blood glucose levels. Such inhibitors, which are used in clinical practice for the treatment of diabetes mellitus, are known to be associated with various adverse gastrointestinal effects. Therefore, it is necessary to identify amylase inhibitors from natural sources with less adverse effects. *Caesalpinia bonducella* aqueous extract inhibited  $\alpha$ -amylase activity by 87.26% [3]. The inhibitory effect on  $\alpha$ -amylase was studied by ethanol extract of *Capparis spinosa*. A concentration of 25 mg/ml of extracts from roots and leaves inhibited  $\alpha$ -amylase by 97.31-98.92% [4].

In vitro  $\alpha$ -amylase inhibitory activity of secondary metabolites isolated from *S. persica* and the chemical composition responsible for its activity were studied. Aqueous extract of *S. persica* highly inhibited  $\alpha$ -amylase activity by 72.39% and had an  $IC_{50}$  value of 376  $\mu$ g/ml compared to acarbose with an inhibitory value of 65.99%. In the analysis of TLC, it was found that there are 15 different compounds [5].

Albidopyrone is synthesized by *Streptomyces sp.*, an actinomycete isolated from sediments at the bottom of the Atlantic Ocean. The chemical structure of albidopyrone is a six-membered aromatic lactone, with a methyl group in the 3rd position, an OH group in the 4th position, and an acetic acid aniline amide residue in the 6th position. Albidopyrone is not an antibiotic, but together with tyrosine kinases, it inhibits the activity of tyrosine phosphate B (TPB), which regulates tyrosine phosphorylation. TPB is the main negative regulator of insulin (controls the phosphor state of the insulin receptor). In this regard, TPB inhibitors are effective agents for the treatment of diabetes [6].

*Botryodiplodia teobromae* is an endophytic fungus isolated from the weed *Euphorbia hirta*. Fungi were treated with 1mM  $AgNO_3$  to synthesize silver nanoparticles. The production of silver nanoparticles was confirmed using the cell filter and surface color

change from colorless to dark brown. Silver nanoparticles synthesized from *Botryodiplodia theobromae* fungi show the highest inhibitory percentage (94%) for  $\alpha$ -amylase [7]. Endophytic fungi were isolated from the leaves of *Artocarpus heterophyllus*. The inhibitory activity of the obtained extracts on  $\alpha$ -amylase was 89%, and 71% on standard acarbose [8].

In the treatment of patients with type 2 diabetes,  $\alpha$ -glucosidase enzyme inhibitors are used. One of them, acarbose, is a pseudo tetrasaccharide that competes with mono- and disaccharides for digestive enzyme binding sites and slows the sequential fermentation of carbohydrates and absorption of carbohydrates in the small intestine [2, 4]. Butacarbose is mainly known as an  $\alpha$ -glucosidase inhibitor and causes side effects such as abdominal distension, flatulence, and diarrhea [9, 10].

In pharmaceuticals, natural products provide greater structural diversity than standard synthetic substances, offering great opportunities for the discovery of new compounds [11]. Lowering blood glucose is an effective tool in improving the survival rate of patients with diabetes. Endophytic fungi isolated from plants can be an alternative source of secondary metabolites for the treatment of diabetes [12].

Endophytic fungi are a hidden treasure trove of chemically important compounds with potential biological activity. Many natural compounds have been isolated from various endophytes [13]. Bioactive compounds produced by endophytic fungi include alkaloids, benzopyranones, quinones, cytochalazins, depsipeptides, enniatins, flavonoids, furandiones, isocoumarins, peptides, polyketones, phenols, hydroquinones, terpenoids, tetralones, and xanthenes. Therefore, endophytes constitute a chemical reserve of new biologically active compounds, which are used in the pharmaceutical and agrochemical industries as antioxidant, antimicrobial, antidiabetic, antiparasitic, antiviral, insecticidal, antibiotic, immunosuppressive, and immunomodulatory agents [14, 15]. Endophytic fungi produce a variety of chemicals to allow them to live in plant cells. Due to their symbiotic relationship, they secrete pharmaceutically important biologically active compounds and enzyme inhibitors. 32 endophytes were isolated from the *Gymnema sylvestre* plant and their inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase of *Fusarium equiseti* endophytic fungus was observed [16]. As an  $\alpha$ -amylase inhibitor, the  $IC_{50}$  value of the methanol extract of *Penicillium oxalicum* was 46.73 mg/ml, and the chloroform extract was 59.20 mg/ml [17]. Extracts from the endophytic fungus *Aspergillus awamori* isolated from *Acacia nilotica* inhibited  $\alpha$ -amylase by 81% and  $\alpha$ -glucosidase by 80% [18].

Secondary metabolites of the endophytic fungus *P. brevicaulis alba-CC200* isolated from *Celosia cristata* were studied as inhibitors of pancreatic  $\alpha$ -

amylase enzyme. The ethylacetate extract of the obtained secondary metabolites showed 93% high inhibitory activity to  $\alpha$ -amylase, and qualitative analysis revealed that the extract contained terpenoid, saponin, glycoside and phenolic components [19]. At the same time, to confirm which of the detected substances exert inhibitory activity, the method consists of elucidating through TLC, column chromatography and qualitative analysis methods.

## II. MATERIAL AND METHODS

### a) Cultivation of endophytic fungi

Both endophytic fungi were grown in the Czapek Dox nutrient medium on orbital shaker Elon 357 (Joanlab, Poland) at 180 rpm, 28°C, and for seven days. The biomass of the cultures was yielded by centrifugation at RS-6 (RHSnab, Russia) at 6,000 rpm and stored at +4°C.

### b) Determination of the inhibitory activity

Each sample obtained after the separation of the methanol fraction on the column was examined for inhibitory activity. The activity of the  $\alpha$ -amylase fractions was determined according to the method used in plant extracts [20]. The starch solution prepared as a substrate in an amount of 1 g/10 ml of water, boiled for 2 min, the sample volume adjusted to 100 ml by distilled water. 100 ml of pancreatic  $\alpha$ -amylase (0.1 M Na-acetate buffer is 13 ml at pH 7.2), 100 mcg of endophyte extract, 2 ml of acetate buffer were incubated for 10 minutes at 30° C for 2 ml of starch prepared from the preparation. The incubation reaction was then stopped and immersed in 10 ml of an aqueous reagent, and the optical density was measured at 630 nm on a SPECOL-1300. To prepare the iodine reagent, 0.5 g of crystalline iodine, 5 g of potassium iodide, and 250 ml of dissolved in water were taken; 2 ml of this reagent was added to 100 ml of 0.1 M HCl to obtain a working solution. The inhibitory activity was expressed by the formula:  $(A0 - At)/A0 \times 100\%$ , where A0 is the absorption of the control sample, and T is the absorption of the experimental sample, respectively. As a comparison drug, acarbose from the commercial drug "Glucobay" (Bayer Pharma AG, Germany) was used.

### c) Phytochemical analysis of extracts of secondary metabolites

Qualitative composition of compounds in extracts of endophytic fungi determined according to Prabhavathi et al. [21].

The tannins and phenolic substances was determined by adding 2-3 drops of 1%  $FeCl_3$  solution to 2 ml of the extract. In the presence of iron ions, tannins give a black-blue or black-green color, and phenols - are purple.

The presence of saponins was established by diluting 1 ml of the extract with 5 ml of hot water (60 °C)

with intensive shaking for 5 minutes until the formation of a persistent foam. The foam volume was maintained for the next 30 minutes.

The terpenoids were determined by mixing 0.5 ml of the extract with 2 ml chloroform and 3 ml of  $H_2SO_4$  (conc.). The formation between the phases of red-brown staining indicates the presence of terpenoids. 2 ml of the extract was mixed with 4 ml of hexane and shaken to determine the terpenoids. At the same time, the separation of the extract into 2 layers was observed. The upper layer was separated, 4 ml of 10% ammonia was added, and the lower layer's color was determined. The purple-pink color indicated the presence of anthraquinones.

The presence of cardiac glycosides was determined by mixing 1 ml of the extract with 1 ml of glacial acetic acid and then adding one drop of 3% ferric chloride in methanol. Then  $H_2SO_4$  (conc.) was added along the tube wall, and the color of the lower layer was determined. Blue-green staining indicated the presence of cardiac glycosides.

To determine flavonoids, a few drops of 20% sodium hydroxide were added to 2 ml of each extract, and the formation of an intense yellow color was observed. Next, a few drops of 70% dilute hydrochloric acid was added, and the yellow color disappeared. The formation and disappearance of yellow color indicate the presence of flavonoids in the sample extract.

The alkaloids were determined by their ability to form compounds insoluble in water with complex iodides, which makes it possible to establish the presence of alkaloids even with their insignificant content. A solution of iodine in potassium iodide (Wagner reagent, Bouchard reagent) with alkaloids form brown, hardly soluble in water precipitates. Five drops of the reagent for precipitation of alkaloids are added to 1 ml of the extract. In the presence of alkaloids, a brown precipitate appears.

#### d) Thin layer chromatography (TLC)

In thin-layer chromatography, components are partitioned between stationary and mobile phases. Separation of the constituent parts moves at different speeds in the TLC plates depending on its polarity in stationary and mobile phases. SIGMA-ALDRICH Silicagel on TLCAL plates of size 10x10 cm were used for TLC. Plates were run in the following eluents: benzene: methanol (40:8) in a standard chromatographic chamber. 25  $\mu$ l of the extracts at a concentration of 25 mg/ml were dropped onto the starting line of the plates. The plates taken from the chromatographic chamber were dried and the following methods were used to determine the separated substances: under ultraviolet light at 254 nm; with iodine vapor in a desiccator; 20% solution of phosphorous-molybdic acid in alcohol; It was sprayed with a 10% solution of  $H_2SO_4$  acid in methanol and dried at 110°C.

#### e) Column chromatography

Partial purification of the extracts was performed with modifications from Kaur et al. The extract was applied to a column (2 x 25 cm) of silica gel (size 100–250). The mobile phase used is chloroform: ethyl acetate: formic acid in a ratio of 5:4:1. Using this solvent system, fractions of 10 mL each were collected and designated as I and II, respectively. Activity was observed in fraction II. The chemical nature of the active fractions was determined using qualitative analysis and TLC methods [22].

Results were the mean of replicate analysis.

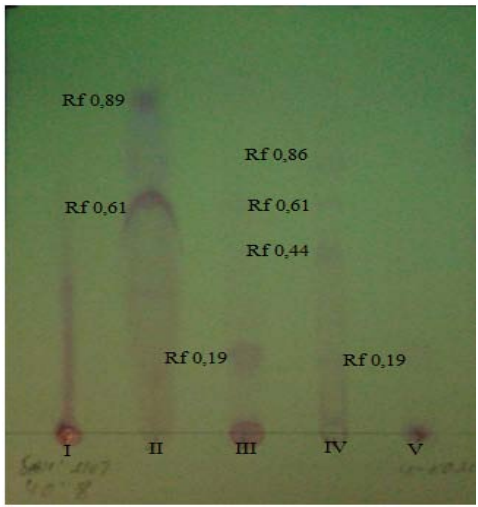
### III. RESULTS AND DISCUSSION

58 compounds that can inhibit  $\alpha$ -amylase and  $\alpha$ -glycosidase enzymes were identified in hyperglycemia. These substances mainly belong to flavones, flavone glycosides, triterpenes, alkaloids, tannins and other polyphenol compounds [23]. Partially purified enzyme inhibitors result in high activity, with maximal inhibitory activity determined based on morphological and molecular analysis [24].

Our results show that ethylacetate extract of *P. brevicaula alba-CC200* contains compounds responsible for the inhibition of amylase activity, so the extract was purified. Five fractions were obtained from the extracts by column chromatography, dissolved in DMSO and tested as an  $\alpha$ -amylase inhibitor. From the data in Table 1, it can be seen that the highest inhibitory activity was 91.2% in fraction II, and the methanol fraction before chromatographic purification was 88.7%. Low inhibitory activity was 58.4% in fraction I, 26% in fraction III, 38.7% in fraction IV, and 12.4% in fraction V.

**Table 1:** Analysis of the inhibitory level of secondary metabolites of the endophytic fungus *P. brevicaula alba-CC200* and the analysis of fractions obtained by column chromatography.


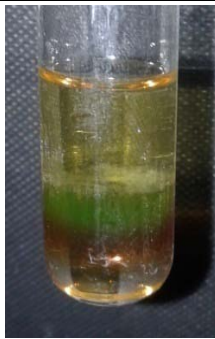
Fractions	$\alpha$ -amylase % inhibitor	Rf
I	58,4	-
II	91,2	0,89
		0,61
III	26	0,19
IV	38,7	0,86
		0,61
		0,44
V	12,4	0,19



Eluent system: benzene:methanol (5:1). The volume of samples used was 25  $\mu$ l. Determining reagent: 20% alcoholic solution of phosphorus-molybdic acid.

Fractions obtained as a result of column chromatography were checked for chemical purity of substances using TLC. When TLC plates were treated with a 20% alcoholic solution of phosphoro-molybdic acid, the spots were colored blue, which is due to the presence of sopenin and triterpenes. Jasleen Kaur et al., partially purified *Alternaria* endophytic fungus and when treated with  $FeCl_3$  on TLC plates, the spots were stained purple, which can be attributed to the characteristic of phenolic compounds in the fraction [25]. Partially purified fraction III obtained from the endophytic fungus *Colletotrichum capsici* was found to have potent antidiabetic activity. In addition, determination by mass

spectrometry showed that compounds in fraction III are mainly fatty acid and phenolic compounds [26]. Terpenoids, formaldehyde and eugenol biologically active substances were detected when ethylacetate extracts of endophytic bacteria isolated from *Mentha piperita* (mint) plant were examined using GC-MS spectrum [27]. Antioxidant, antidiabetic and anticholinesterase activity of biologically important phytochemicals contained in the methanol extract of *C. uredinicola* endophytic fungi was determined in vitro. The qualitative analysis of *C. uredinicola* extract shows the presence of flavonoids, tannins, alkaloids, glycosides, phenols, terpenoids and coumarins [28].

<i>P. brevicaula alba-CC200</i>			
Fraction	$\alpha$ -amylase % inhibitor	Terpenoids	Cardiac glycosides
II	91,2		

**Figure 1:** Qualitative analysis of fraction II obtained by column chromatography

Also, fraction II with high inhibitory activity was tested by qualitative reactions for several chemical classes. The presence of cardiac glycosides and

terpenoids was determined in the fraction with 91.2%  $\alpha$ -amylase inhibitor. Figure 1.

Many studies suggest the use of triterpenes in the prevention of diabetic complications such as nephropathy, embryopathy, neuropathy or wound healing [29]. For example, oleanolic acid, betulinic acid and ursodeoxycholic acid are strong inhibitors of TGR5 receptors involved in energy metabolism [30]. The n-hexane fraction with the highest inhibitory activity of the plant *Nuxia oppositifolia* confirmed the presence of 3-oxolupenal and cathononic acid when examined using GC-MS analysis [31]. Many reports attribute the  $\alpha$ -amylase inhibitory effect of the extracts to the terpenoids present in the extracts [32, 33].

#### IV. CONCLUSION

Fraction II of the endophytic fungus *P. brevicaula alba - CC200*, purified by column chromatography, inhibited  $\alpha$ -amylase by 91.2%. TLC and quality analysis showed that the high inhibitory activity of fraction II is due to the content of cardiac glycosides and terpenoids. These natural bioactive compounds can inhibit  $\alpha$ -amylase enzyme activity and regulate blood glucose levels. It can be an alternative source for hyperglycemic compounds.

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Fellows can publish articles (limited) without any fees. Also, they can earn up to 70% of sales proceeds from the sale of reference/review books/literature/publishing of research paper. The FMRC member can decide its price and we can help in making the right decision.

Exclusive

Financial

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Credibility

Exclusive

Reputation

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## ASSOCIATE OF MEDICAL RESEARCH COUNCIL

ASSOCIATE OF MEDICAL RESEARCH COUNCIL is the membership of Global Journals awarded to individuals that the Open Association of Research Society judges to have made a 'substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Associate membership can later be promoted to Fellow Membership. Associates are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Associate Members.



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### TO THE INSTITUTION

#### GET LETTER OF APPRECIATION

Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.



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#### GET ACCESS TO A CLOSED NETWORK

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Career

Credibility

Exclusive

Reputation



### CERTIFICATE

#### CERTIFICATE, LOR AND LASER-MOMENTO

Associates receive a printed copy of a certificate signed by our Chief Author that may be used for academic purposes and a personal recommendation letter to the dean of member's university.

Career

Credibility

Exclusive

Reputation



### DESIGNATION

#### GET HONORED TITLE OF MEMBERSHIP

Associates can use the honored title of membership. The "AMRC" is an honored title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., AMRC or William Walldroff, M.S., AMRC.

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Credibility

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Career

Credibility

Reputation

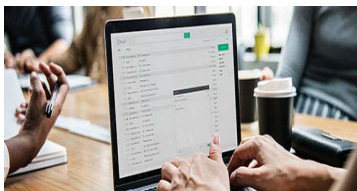
## FUTURE WORK

### GET DISCOUNTS ON THE FUTURE PUBLICATIONS

Associates receive discounts on future publications with Global Journals up to 30%. Through our recommendation programs, members also receive discounts on publications made with OARS affiliated organizations.

Career

Financial



## GJ ACCOUNT

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Career

Credibility

Reputation



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Financial

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### ORGANIZE SEMINAR/CONFERENCE

Associates are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

Career

Credibility

Financial

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All associates receive the early invitations to all the symposiums, seminars, conferences and webinars hosted by Global Journals in their subject.

Exclusive





## PUBLISHING ARTICLES & BOOKS

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Exclusive

Financial

## REVIEWERS

### GET A REMUNERATION OF 15% OF AUTHOR FEES

Associate members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

Financial

## AND MUCH MORE

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ASSOCIATE	FELLOW	RESEARCH GROUP	BASIC
<p>\$4800 lifetime designation</p> <hr/> <p>Certificate, LoR and Momento 2 discounted publishing/year Gradation of Research 10 research contacts/day 1 GB Cloud Storage GJ Community Access</p>	<p>\$6800 lifetime designation</p> <hr/> <p>Certificate, LoR and Momento Unlimited discounted publishing/year Gradation of Research Unlimited research contacts/day 5 GB Cloud Storage Online Presense Assistance GJ Community Access</p>	<p>\$12500.00 organizational</p> <hr/> <p>Certificates, LoRs and Momentos Unlimited free publishing/year Gradation of Research Unlimited research contacts/day Unlimited Cloud Storage Online Presense Assistance GJ Community Access</p>	<p>APC per article</p> <hr/> <p>GJ Community Access</p>



# PREFERRED AUTHOR GUIDELINES

## **We accept the manuscript submissions in any standard (generic) format.**

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from <https://globaljournals.org/Template>

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at [submit@globaljournals.org](mailto:submit@globaljournals.org) or get in touch with [chiefeditor@globaljournals.org](mailto:chiefeditor@globaljournals.org) if they wish to send the abstract before submission.

## BEFORE AND DURING SUBMISSION

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct*, along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

## **Declaration of Conflicts of Interest**

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

## POLICY ON PLAGIARISM

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

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- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures



- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

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1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

### Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

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### Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

### Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

### Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

## PREPARING YOUR MANUSCRIPT

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

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.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

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



## FORMAT STRUCTURE

***It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.***

All manuscripts submitted to Global Journals should include:

### **Title**

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

### **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.



## Figures

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).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

### 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.



**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.

**9. Produce good diagrams of your own:** Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

**10. Use proper verb tense:** Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

**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.

**21. Adding unnecessary information:** Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

**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 through 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.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

### **General style:**

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

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





### *Mistakes to avoid:*

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- 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.

### **Title page:**

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.



**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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<i>Introduction</i>	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
<i>Methods and Procedures</i>	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
<i>Result</i>	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
<i>Discussion</i>	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
<i>References</i>	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring



# INDEX

---

---

## **A**

Aspiration · 1, 2, 4, 5, 8, 9, 10, 12, 18, 19

---

## **D**

Discordance · 5, 8

---

## **E**

Elucidating · 23

---

## **F**

Flatulence · 23

---

## **M**

Meticulous · 8

---

## **N**

Nodules · 1, 2, 3, 6, 7, 9, 10, 18, 19

---

## **P**

Precipitates · 24

---

## **R**

Replicate · 25



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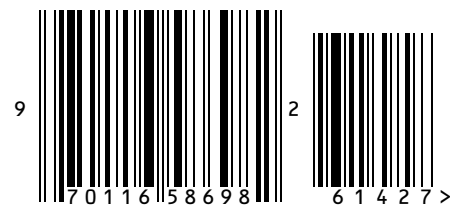


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