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Menorracy of Polymorphism

Effect of Monovalent Copper Ions

Highlights

Microorganisms in Meat Products

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Discovering Thoughts, Inventing Future

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Association with the Development and Menorracy of Polymorphism rs2046934 of the P2ry12 Gene in Patients with Dysaggregation Thrombocytopathies

By Shakhnoza G. Sabirova

Abstract- The results of studying the peculiarities of the P2RY12 gene polymorphism (rs2046934) revealed in the main group of road traffic accidents an increase in the proportion of the unfavorable allele A by 2.24 times ($\chi 2=3.61$; $P=0.06$; $OR=2.24$) in relation to the control, which indicates the presence of a tendency towards the risk of developing disaggregated thrombocytopathies. In addition, there was an increase among patients with NDTP of the mutant genotype A / A ($\chi 2=3.04$; $P=0.08$). Indicates a tendency towards an increased risk of development and associative relationship with the clinic (namely with menorrhagia) ($\chi 2=5.6$; $P=0.02$; $OR=4.3$) of this disease.

Keywords: polymorphism, allele, unfavorable, genotype, risk of development, menorrhagia.

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Association with the Development and Menorracy of Polymorphism rs2046934 of the P2RY12 Gene in Patients with Dysaggregation Thrombocytopathies

Shakhnoza G. Sabirova

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Keywords: polymorphism, allele, unfavorable, genotype, risk of development, menorrhagia.

I. Introduction

Among the pathologies, disorders of the hemostasis system, that is, hemorrhagic diathesis, 70-80% are thrombocytopathies and thrombocytopenia [1,2,9]. Thrombocytopathies are a group of diseases in the pathogenesis, which is functional disorders and qualitative platelet inferiority. As everyone knows, thrombocytopathies can be both hereditary and acquired. Among the hereditary forms of thrombocytopathies, the most common is Thrombasthenia Glanzmann's disease, in which the disorder occurs due to the aggregation function of platelets, that is, hereditary disaggregation thrombocytopathy (HDTP) [3,4,5,8].

A number of scientific studies are being carried out in the world aimed at studying various aspects of the mechanisms of development and formation of TP [13,14,15]. However, despite the progress achieved in this area, many of their sides, in particular with disaggregated forms of thrombocytopathies(DTP) (contribution of molecular genetic polymorphisms, their relationship with clinical manifestations) to this day remain an urgent problem [11,12], including among the Uzbek ethnic group. We conducted studies to assess the correlation between the clinical manifestations of dysaggregated thrombocytopathies and the molecular genetic markers of platelet dysfunction P2RY12, which is of particular importance today.

The aim of the study is to determine the associative relationship of clinical manifestations with the genetic marker P2RY12 (rs2046934) in patients with disaggregated thrombocytopathies of the Uzbek ethnic group.

II. Material and Research Methods

A comprehensive examination of 90 unrelated patients was carried out (the main group of road accidents, men - 30 (33.3%), women - 60 (66.7%) among which the 1st subgroup consisted of patients with HDTP (n=50)(Thrombasthenia Glanzmann) and 2nd subgroup - patients with ADTP (n=40), who were under observation and inpatient treatment in the clinic of the Research Institute of Hematology and Blood Transfusion of the Ministry of Health of the Republic of Uzbekistan. The selection of patients was carried out by the method of random sampling as they approached. The median age of patients in the main group of road traffic accidents was 31.4 ± 1.2 years. The control group consisted of 48 conditionally healthy unrelated persons with no history of hemostasis pathology, which matched the sex and age of the examined main group of patients.

The research methods were clinical and molecular genetic studies and statistical methods.

Clinical methods included collection of complaints, anamnesis and an objective examination of the patient.

As a material for the molecular genetic study of polymorphic variants of the platelet receptor gene P2RY12 (rs2046934), we used the venous blood of patients with road traffic accidents, as well as conditionally healthy individuals. Genotyping was performed using polymerase chain reaction (PCR) followed by analysis of restriction fragment length polymorphism (RFLP) of PCR products. Genomic DNA was isolated from the nuclei of leukocytes of venous blood stabilized with 0.5 M EDTA, after which its concentration was measured on a spectrophotometer, and amplification was performed. The specificity and the

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number of amplified fragments were checked by agarose gel electrophoresis. Amplification and restriction products were separated in 6.0-10.0% in 2.0-3.0% agarose or polyacrylamide gels. For the detection of amplification products in agarose gel, we used chambers for horizontal electrophoresis "Helikon" ("DNA-Technology"). The patient's genotype was determined in accordance with the set of DNA fragments identified in the gel as a result of PCR-RFLP analysis.

Electropherogram detection of rs2046934 polymorphism of the P2RY12 gene in the control group and in patients with road traffic accidents (see Figure 1).

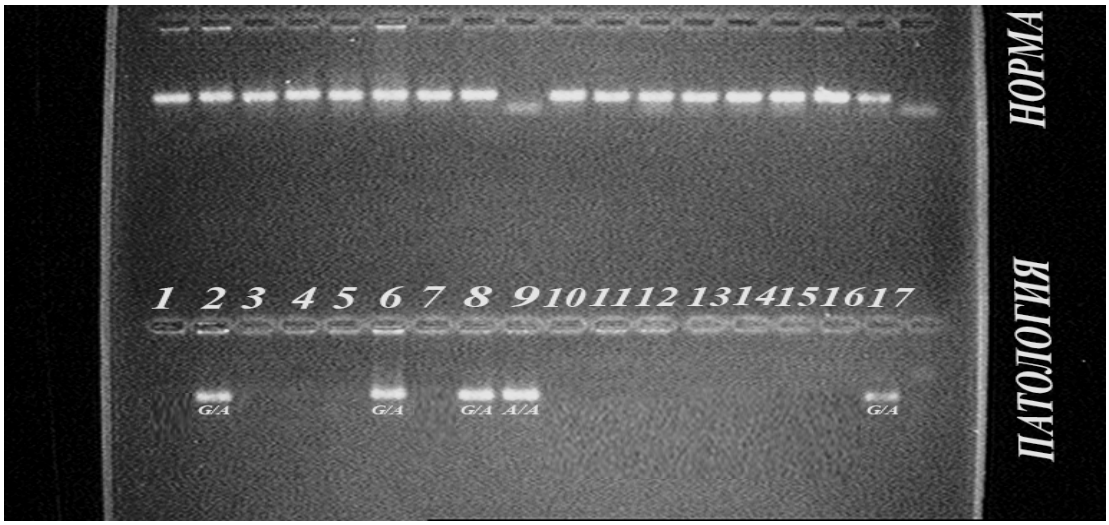


Figure 1: The specificity and the numbers of amplified fragments were checked by electrophoresis in 4% agarose gel.

a) Statistical analyses

Statistical processing of the obtained results was carried out on a personal computer using the programs "OpenEpi 2009, Version 2.3". To determine the differences in the frequency of occurrence of genotypes between the study groups, Fisher's exact test was used. The correspondence of the distribution of genotypes in the examined groups to the canonical distribution of Hardy-Weinberg was assessed using the $\chi 2$ test. Differences between groups were statistically significant at $p < 0.05$.

III. Results and Discussions

Studying the clinical manifestations of the disease, it was revealed, that road traffic accidents, regardless of hereditary or acquired nature, are mainly manifested by nosebleeds (59.0%) and petechial rash on the skin (38.0%). However, at the same time, it is important to note that NDTP proceeds with more pronounced hemorrhagic manifestations, observed in 56.0% of cases already in preschool and 44.0% at school age. Whereas ADTP in the main (70.0% of cases), manifested itself in the adult period of life. Along with this, with increasing age, the DTP acquires a more severe course, which is confirmed by the significantly expressed and increase in the number of hemorrhagic clinical manifestations of the disease ($p > 0.05$). In particular, road traffic accident patients with a median age of 29.30 ± 1.79 years more often had one clinical symptom, patients with a median age of 32.66 ± 2.50 had two symptoms, while patients with a median age of 34.27 ± 5.09 the disease manifested itself with three symptoms.

The results of studying the peculiarities of the P2RY12 gene polymorphism (rs2046934) revealed in the main group of road traffic accidents an increase in the proportion of the unfavorable allele A by 2.24 times ($\chi 2=3.61$; $P=0.06$; $OR=2.24$) in relation to the control, which indicates the presence of a tendency towards the risk of developing this disease. At the same time, a statistically insignificant 1.57-fold increase in the frequency of the heterozygous G/A genotype was observed in the group of patients ($\chi 2=0.88$; $p=0.35$; $OR=1.57$; 95% CI=0.61-4.03). In addition, the increase among patients with road traffic accidents of the mutant genotype A/A ($\chi 2=3.04$; $P=0.08$) indicates the presence of a tendency to increase the risk of developing the disease (see Table 1).

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Table 1: Frequency distribution of alleles and genotypes of rs2046934 polymorphism of the P2RY12 gene in patient and control groups

№	Group	n	Allele frequency				Genotype distribution frequency					
			G		A		G/G		G/A		A/A	
			n	%	N	%	n	%	n	%	n	%
1	Main group DTP	71	118	83,1	24	16,9	51	71,8	16	22,5	4	5,6
A	HDTP	39	63	80,8	15	19,2	27	69,2	9	23,1	3	7,7
B	ADTP	32	55	85,9	9	14,1	24	75,0	7	21,9	1	3,1
2	Control group	48	88	91.7	8	8.3	40	83,3	8	16,7	0	0

The study of the associative relationship between the carriage of an unfavorable allele A and the risk of road traffic accidents showed that in the subgroup of patients with HDTP, this allele significantly increases the risk of developing the disease by 2.62 times ($\chi2=4.46$; $P=0.035$; OR=2.62; 95% CI: 1.05-6.55). In the subgroup of ADTP patients in carriers of the unfavorable allele A, the risk of developing the disease increased by 1.8 times, but this was not significant ($\chi2=1.33$; $P=0.25$; OR=1.8; 95% CI: 0.66-4.94).

The study of the associative relationship between the carriage of the heterozygous genotype G/A and the risk of developing the disease revealed a statistically insignificant increase in the risk of developing HRTP by 1.67 times ($\chi2 <3.8$; $P> 0.05$; OR=1.67; 95% CI: 0.57-4.86)and ADTP by 1.46 times ($\chi2 <3.8$; $P> 0.05$; OR=1.46; 95% CI: 0.47-4.53). With regard to the A / A mutant genotype, a statistically significant association with the risk of developing the disease was found in the subgroup of patients with HDTP ($\chi2=4.18$; $P=0.04$) and insignificant in the subgroup of patients with ADTP ($\chi2=1.63$; $P=0.20$) (see Figure 2).

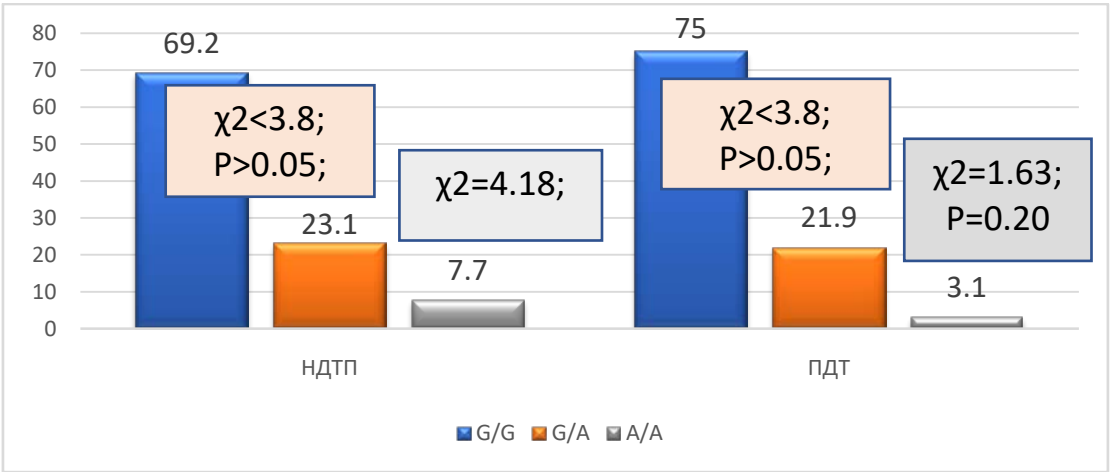


Figure 2: Associative relationships between the carriage of the genotypes of the P2RY12 gene polymorphism (rs2046934) and the development of HDTP and ADTP

The results of a comparative analysis of the frequency and structure of carriage of the polymorphism of the genes of the platelet receptor P2RY12 (rs2046934) in patients with NDTP and in relatively healthy individuals allowed us to establish the involvement of the mutant genotype A / A ($\chi2=4.18$; $P=0.04$) of the P2RY12 polymorphism (rs2046934) in the formation of NDTP in individuals Uzbek ethnic group.

Thus, the results showed that the P2RY12 gene polymorphism (rs2046934) is an independent marker of an increased risk of developing a hereditary form of dysaggregation thrombocytopathy, and does not act as an independent genetic marker in the development of the acquired form of disaggregated thrombocytopathy in persons of the Uzbek ethnic group.

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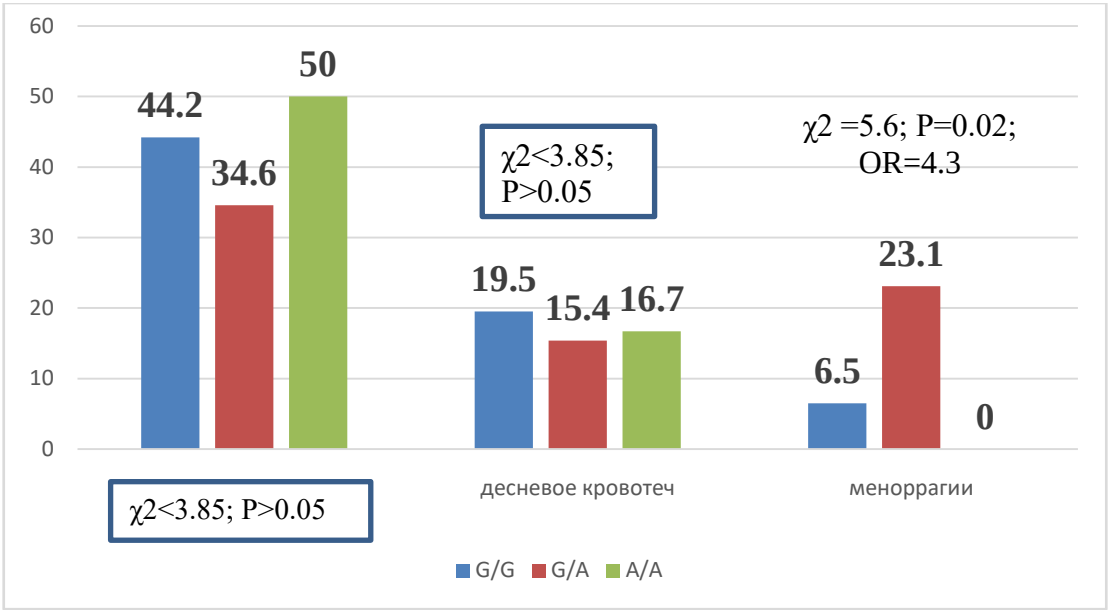


Figure 3: Association of the incidence of nasal, gingival and menorrhagia bleeding with the carriage of unfavorable genotypes of the rs2046934 polymorphism of the P2RY12 gene.

At the same time, we studied the presence of a possible association of the molecular genetic marker P2RY12 of platelet dysfunction with the clinical manifestations of road traffic accidents. The study showed that there was a significant relationship between the carriage of an unfavorable heterozygous G / A genotype of the rs2046934 polymorphism of the P2RY12 gene in patients with a hereditary form of road traffic accidents and the frequency of menorrhagias ($\chi2=5.6$; $P=0.02$; $OR=4.3$) and the absence of a significant association with respect to other clinical signs with carriage unfavorable genotypes of the studied genes ($\chi2 <3.85$; $P>0.05$) (see Figure 3).

IV. Conclusions

It is known that the platelet receptor P2RY12, being bound to the G-protein, is responsible for the enhancement and completion of platelet aggregation by inhibiting adenylate cyclase, leading to limitation of the activity of protein kinase A by dephosphorylation of phosphoprotein and activation of phosphoinositol-3-kinase and small guanosine triphosphotics. A genetic defect or exogenous inhibition of the P2RY12 platelet receptor leads to a pronounced impairment of platelet aggregation [6,7,10].

It was found that the genetic predisposition to the development of disaggregation thrombocytopathies for the rs2046934 polymorphism of the P2RY12 gene is reliably associated with the functionally unfavorable homozygous genotype A/A, which is expressed especially in patients with hereditary disaggregation thrombocytopathies, however, carriers of an unfavorable heterozygous genotype have an extremely low risk of developing aggregation disorders.

Thus, as a result of the study, it was established that the development of road traffic accidents is genetically determined. A significant association of the risk of menorrhagia in patients with NDTP with polymorphism of the platelet receptor gene P2RY12 (rs2046934), which is involved in the main pathogenetic mechanisms of platelet dysfunction, was revealed. The results obtained make it possible to use this genetic marker as a prognostic factor for the formation of hereditary road traffic accidents and the identification of risk groups for the development of the disease in persons of the Uzbek ethnic group.

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Controle De Qualidade De Cápsulas De Ibuprofeno De Farmácias De Manipulação De Manaus- Am

By Danilo M. Maciel, Maykon P. G. Marinho, Wemelly C. A. Naziazeno, Rodrigo Queiroz de Lima & Marcos Túlio da Silva

Abstract- Nowadays, the population has been searching more and more for the services of the handling pharmacies, being them a form of access to personalized medicines. With this, it was sought to investigate the reliability of manipulated capsules in different pharmacies in the city of Manaus. The project is an analytical and observational research, of the transversal type, quantitative where the determination of the average weight was analyzed, as well as the test of disintegration of the samples. The criteria and specifications contained in the 6th edition of the Brazilian Pharmacopoeia were used, the data obtained from the tests of average weight were calculated the limits of variances and standard deviations for the tabulation in Excel® spreadsheet (Microsoft 2013), finally tables were assembled for the pharmacies. The results were examined and compared with the specifications pre-established in the 6th edition of the Brazilian Pharmacopoeia.

Keywords: drug quality control, disintegration, ibuprofen, brazilian pharmacopoeia.

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Controle De Qualidade De Cápsulas De Ibuprofeno De Farmácias De Manipulação De Manaus- Am

Danilo M. Maciel [α], Maykon P. G. Marinho [σ], Wemelly C. A. Naziazeno [ρ], Rodrigo Queiroz de Lima [ω] & Marcos Túlio da Silva [¥]

Resumo- Nos dias atuais, a população tem buscado cada vez mais os serviços das farmácias de manipulação, sendo elas, uma forma de acesso a medicamentos personalizados. Com isso, buscou-se investigar a confiabilidade de cápsulas manipuladas em diferentes farmácias da cidade de Manaus. O projeto trata-se de uma pesquisa analítica e observacional, do tipo transversal, quantitativo onde se analisou a determinação do peso médio, bem como o teste de desintegração das amostras. Foram utilizados os critérios e especificações contidos na 6ª edição da Farmacopeia Brasileira, dos dados obtidos dos ensaios de peso médio foram calculados os limites de variâncias e desvios padrão para a tabulação em planilha do Excel® (Microsoft 2013), por fim foram montadas tabelas para as farmácias. Os resultados foram examinados e comparados com as especificações preestabelecidas na 6ª edição da Farmacopeia Brasileira. A pesquisa indicou, com a análise dos dados, a taxa de qualidade das cápsulas manipuladas em farmácias de manipulação de Manaus, em relação ao seu tempo de desintegração e sua conformidade no peso médio, o que demonstroua qualidade das cápsulas analisadas.

Palavras-chaves: controle de qualidade de medicamentos, desintegração, ibuprofeno, Farmacopeia Brasileira.

Abstract- Nowadays, the population has been searching more and more for the services of the handling pharmacies, being them a form of access to personalized medicines. With this, it was sought to investigate the reliability of manipulated capsules in different pharmacies in the city of Manaus. The project is an analytical and observational research, of the transversal type, quantitative where the determination of the average weight was analyzed, as well as the test of disintegration of the samples. The criteria and specifications contained in the 6th edition of the Brazilian Pharmacopoeia were used, the data obtained from the tests of average weight were calculated the limits of variances and standard deviations for the tabulation in Excel® spreadsheet (Microsoft 2013), finally tables were assembled for the pharmacies. The results were examined and compared with the specifications pre-established in the 6th edition of the Brazilian Pharmacopoeia. The research indicated, with the data analysis, the quality rate of the capsules handled in handling pharmacies in Manaus, in relation to their disintegration time and their conformity to the average weight, which demonstrated the quality of the capsules analyzed.

Keywords: drug quality control, disintegration, ibuprofen, brazilian pharmacopoeia.

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I. Introdução

Nos dias atuais, a população tem buscado cada vez mais os serviços das farmácias de manipulação, sendo elas, um acesso a uma forma de personalização de apresentações de medicamentos[1]. Logo, a farmácia de manipulação tem devida importância e é de grande interesse da população, que viabiliza o uso racional de medicamentos, um dos propósitos da Política Nacional de Medicamentos (PNM)[2].

A Agencia Nacional de Vigilância Sanitária (ANVISA), observou a necessidade de um controle e regularização das farmácias de manipulação, tendo no ano de 2000 a publicação da primeira resolução relacionada às boas práticas de manipulação em farmácia[2,3]. A RDC67 de 08 de outubro de 2007 é a mais atual e vigente que regulamenta as Boas Práticas de Manipulação de Preparações Magistrais e Oficinais para Uso Humano em farmácias[4].

A RDC 67/2007, descreve os requisitos mínimos para a realização das atividades das farmácias de manipulação, desde suas instalações até a atenção farmacêutica, abrangendo todos os seus setores, tendo em vista a garantia da qualidade, categoriza também as farmácias por grupo, classificando por atividades/natureza dos insumos manipulados[4].

A Farmacopeia Brasileira, associada a outros compêndios oficiais (Farmacopeia Europeia, Britânica, Americana, entre outras) é o conjunto de textos incumbido de dispor as especificações de qualidade, pureza e autenticidade mínimas de produtos farmacêuticos os quais são submetidos à fiscalização da vigilância sanitária. Estando a Farmacopeia da República Federativa do Brasil na sua 6ª edição, atualiza em agosto de 2019[5].

Cápsula é uma das formas farmacêuticas mais utilizadas no ramo das farmácias de manipulação, sendo utilizadas na produção mais especificamente, as cápsulas gelatinosas duras. Para serem

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comercializadas, as cápsulas, devem estar no mínimo dentro dos padrões e especificações nos ensaios de descrição, aspecto, características organolépticas, peso médio (devendo ser calculados, o desvio padrão e o coeficiente de variação em relação ao peso médio). Como forma de monitoramento do controle de qualidade deve-se ainda realizar análises de teor e uniformidade do conteúdo das cápsulas[6].

O teste de desintegração possibilita investigar se comprimidos e cápsulas se desintegram dentro do limite de tempo especificado na Farmacopeia, quando seis unidades do mesmo lote são submetidas ao desintegrador, sob condições experimentais descritas. O teste se aplica a comprimidos não revestidos, revestidos com filme ou com revestimento açucarado (drágeas), comprimidos com revestimento entérico, comprimidos sublinguais, comprimidos solúveis, comprimidos dispersíveis, cápsulas duras e cápsulas moles. Pode ser aposto a comprimidos mastigáveis; nesse caso, as condições e critérios de avaliação constarão na monografia individual. O ensaio não se aplica a pastilhas, comprimidos ou cápsulas de liberação controlada (prolongada). A desintegração é determinada, para os fins desse teste, como o estado no qual nenhum resíduo das unidades testadas (cápsulas ou comprimidos) encontre-se na tela metálica do aparelho de desintegração, salvo fragmentos insolúveis de revestimento de comprimidos ou invólucros de cápsulas. Consideram-se, também, como desintegradas as unidades que durante o teste se transformam em massa viscosa, desde que não apresentem núcleo tangível[7].

O teste de peso médio se aplica a formas farmacêuticas sólidas em dose unitária (comprimidos não revestidos, comprimidos revestidos, pastilhas, cápsulas duras e moles e supositórios), formas farmacêuticas sólidas acomodadas em recipientes para dose unitária (pós-estéreis, pós-liofilizados, pós para injetáveis e pós para reconstituição de uso oral) e as formas farmacêuticas sólidas e semissólidas acomodadas em recipientes para porções múltiplas (granulados, pós, géis, cremes, pomadas e pós para reconstituição)[7].

Resultados negativos nos testes de controle de qualidade desses medicamentos mostram que os procedimentos de manipulação precisam passar por uma revisão, que envolve: análise de matéria-prima, processo de pesagem, mistura dos pós, processo de encapsulação e armazenamento de formulações magistrais, visando obter produtos com qualidade, atestando a eficácia e segurança do tratamento[8].

O controle de qualidade é uma importante ferramenta para assegurar a eficácia do medicamento e segurança do paciente que utilizará dessa medicação. É possível notar queainda há lacunas no que diz respeito controle de qualidade de medicamentos manipulados. O peso médio e o teste de desintegração, dentre outros; são ensaios descritos para o controle de cápsulas gelatinosas duras, necessários para comprovar a conformidade e eficácia dos medicamentos. Tendo em vista os pontos citados, se fez preciso a realização de estudos da qualidade dos medicamentos manipulados, a fim de verificar sua eficácia.

Deste modo, o objetivo deste estudo foi realizar o peso médio e o teste de desintegração de cápsulas manipuladas em diferentes farmácias da cidade de Manaus.

II. Metodologia

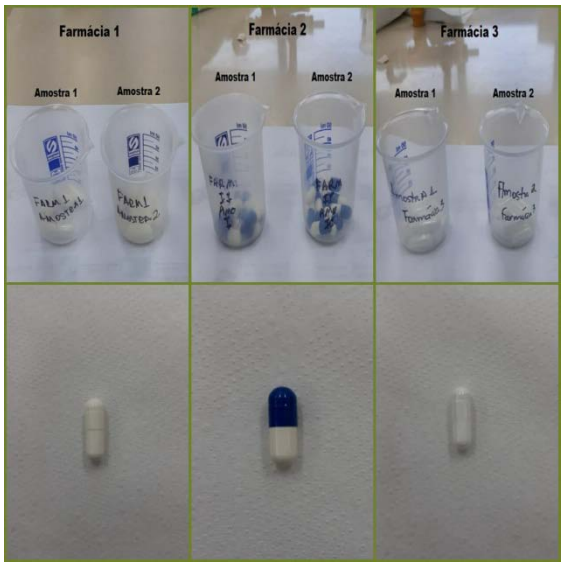
1. Delineamento experimental

As análises do controle de qualidade das cápsulas foram realizadas no Laboratório Mini-indústria do Centro Universitário do Norte (UNINORTE), sob orientação do professor MSc. Marcos Túlio e Rodrigo Queiroz de Lima.

Foram avaliadas cápsulas de ibuprofenode 300mg (Medicamento isento de prescrição-MIP) manipuladas por três farmácias para verificar se as mesmas encontravam-se dentro das especificações da legislação vigente.

As cápsulas manipuladas de ibuprofeno 300mg, foram analisadas nos testes de peso médio e desintegração (Figura 1).

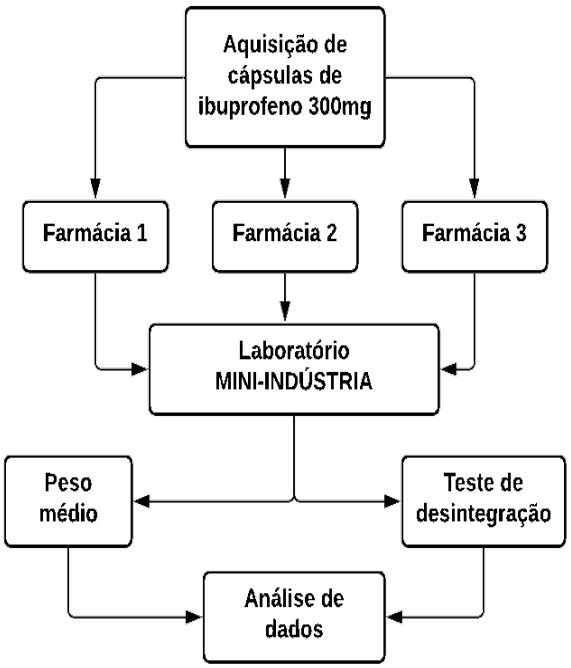
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Fonte: Próprios autores

Figura 1: Cápsulas de ibuprofeno das Farmácias 1, 2e 3 respectivamente.

A aquisição das cápsulas de ibuprofeno 300mg foi feita por meio da compra sem receita, por se tratar de um MIP (Medicamento isento de prescrição), em três diferentes farmácias de manipulação do município de Manaus, nomeadas Farmácia 1, Farmácia 2 e Farmácia 3 (Figura 2). As farmácias de manipulação foram escolhidas devido ao fato de serem consideradas as maiores da cidade de Manaus, consequentemente as mais procuradas. Foram adquiridas 60cápsulas (2 embalagens com 30 cápsulas cada) de cada farmácia, nomeadas amostras 1 e 2 para cada farmácia.



Fonte: Próprios autores

Figura 2: Fluxograma – Delineamento Experimental

2. Critérios de inclusão e exclusão

Inclusão: Cápsulas manipuladas; cápsulas de farmácias de manipulação do município de Manaus; forma farmacêutica cápsula.

Exclusão: Cápsulas industrializadas; cápsulas de farmácias de manipulação de outros municípios que não sejam Manaus; formas farmacêuticas que não sejam cápsulas.

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3. Procedimento metodológico

Peso médio

A determinação de peso médio foi realizada de acordo com os critérios e especificações contidas na 6ª Farmacopeia Brasileira (2019). Vinte cápsulas das amostras 1 e 2 de cada farmácia respectivamente, foram pesadas individualmente e, após remoção do conteúdo, foram novamente pesadas. Utilizaram-se os valores obtidos para calcular o conteúdo de cada cápsula como sendo a diferença entre a cápsula com conteúdo e a vazia. Em seguida realizou-se o cálculo de média e da determinação da variação percentual do conteúdo das cápsulas em relação à média. A análise de peso médio foi feita em duplicata.

Desintegração

Para o teste de desintegração das amostras 1 e 2 de cada farmácia respectivamente, utilizaram-se os critérios e especificações contidos na 6ª Farmacopeia Brasileira (2019). Foram usadas seis cápsulas, que passaram pelo processo dedesintegração em equipamento da Ethiktechnology, por imersão em meio composto por água destilada, segundo a monografia do ibuprofeno, a uma temperatura de 37°C (podendo variar 1º C para mais e para menos), por no máximo 30 minutos. Ao final do teste verificou-se se as cápsulas haviam se desintegrado e o tempo de desintegração foi anotado. A análise de desintegração foi feita em duplicata.

4. Análise de dados

Os dados de peso médio e desintegração de todas as farmácias foram plotados em planilha de Microsoft Excel®2013 para análise estatística.

III. Resultados Ediscussão

Os resultados do teste de peso médio das cápsulas de ibuprofeno, correspondentes às formulações das farmácias 1, 2 e 3, respectivamente, estão apresentados na Tabela 1.

Para as farmácias 1 e 2 o limite de variância tolerável foi de ±7,5%, devido ao peso médio estar acima de 300mg, já para a farmácia 3, o limite de variância tolerável foi de ±10%, pois o peso médio esteve abaixo de 300mg.

De acordo com a Farmacopeia Brasileira (2019)5, no máximo 2 cápsulas podem estar fora dos limites descritos para que sejam aprovadas. Porém, nenhuma poderá estar acima ou abaixo do dobro das porcentagens indicadas. Nenhuma das amostras analisadas ficou fora dos limites especificados, demonstrando homogeneidade de peso.

Tabela 1: Teste de peso médio das amostras analisadas

AMOSTRAS	REPLICATA	PESO (mg)	DESVIO PADRÃO	DESVIO INDIVIDUAL (mg)	RESULTADO PARA O LIMITE DE VARIÂNCIA
FARMÁCIA 1	01	374,46	3,40	346,37 a 402,54	De acordo
	02	361,83	6,82	334,69 a 388,97	De acordo
FARMÁCIA 2	01	406,96	14,86	376,44 a 437,48	De acordo
	02	415,13	42,40	383,99 a 446,26	De acordo
FARMÁCIA 3	01	297,04	7,36	267,34 a 326,74	De acordo
	02	298,06	7,44	268,25 a 327,86	De acordo

As análises definidas pela legislação vigente para preparações magistrais e oficinais sólidas (descrição, aspecto, caracteres organolépticos e peso médio) não são suficientes por não atestarem quanto à homogeneidade do princípio ativo no medicamento, de forma direta, mas apenas quanto à uniformidade do preenchimento das cápsulas9.

Porém, observou-se que o peso médio da amostra 1 (297,04mg) e amostra 2 (298,06mg), ambos da farmácia 3, estão abaixo da concentração do princípio ativo (300mg), o que significa que, as referidas amostras, não possuem o conteúdo mínimo declarado de 300mg de ibuprofeno. Ao fazer uma simples subtração, nota-se que há uma falta de 2,96mg (na amostra 1) e 1,94mg (na amostra 2) de conteúdo para que as mesmas completem o mínimo esperado de fármaco, que é 300mg. Para este caso, recomenda-se aplicar o teste de teor de fármaco, para determinar aquantidade exatadeprincípio ativo presente nas amostras.

Segundo um estudo10 realizado com cápsulas manipuladas de atenolol, quanto menor for o desvio padrão, mais homogênea as amostras estão, indicando a uniformidade durante a produção. Ao avaliar o desvio padrão da farmácia 2 (amostra 2), observou-se que o mesmo estava um pouco acima das demais amostras.

Esses resultados indicam a necessidade de revisão dos procedimentos de manipulação, queenvolvem análise de matéria-prima, processo de pesagem, mistura dos pós, processo de encapsulação e armazenamento de formulações magistrais, visando obter produtos com qualidade, garantindo a eficácia e segurança do tratamento[8].

O resultado obtido vai ao encontro de um estudo11realizado em 2010, que avaliou a qualidade de cápsulas de ibuprofeno de 100 e 200 mg. No referido estudo verificou-se semelhança no peso médio entre as amostras analisadas (apósrealizar um cálculo de proporção), tendo todas as amostras aprovadas. Foi possível encontrar diferença, porém não significativa, na

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distribuição de peso de algumas unidades dos dois estudos.

Por tanto, todas as amostras analisadas encontram-se em conformidade com o preconizado pela Farmacopeia para o teste de peso médio.

Para o teste de desintegração, as amostras das farmácias 1, 2 e 3 atenderam às especificações estabelecidas pela Farmacopeia Brasileira (2019)5, ou seja, as cápsulas estavam completamente desintegradas ao final de 30 minutos. Em um estudo12em 2019, que avaliou a qualidade de cápsulas manipuladas de fluconazol, foi possível notar certa semelhança no teste de desintegração, tendo como menor tempo 1,34min e 5,41min para o maior tempo, tendo todas suas amostras aprovadas no devido teste.

Tabela 2: Teste de desintegração em água a 37°c

AMOSTRAS	TEMPO DE DESINTEGRAÇÃO (MIN.)	RESUL-TADO
FARMÁCIA 1	03:19 a 08:36	De acordo
FARMÁCIA 2	05:05 a 07:41	De acordo
FARMÁCIA 3	04:00 a 06:29	De acordo

Fonte: Próprios autores.

No teste de desintegração das cápsulas apresentadas na Tabela 2, foi observado que todas as amostras foram desintegradas no tempo preconizado na Farmacopeia Brasileira (2019)5, não havendo diferenças significativas no tempo de desintegração das cápsulas das farmácias 1, 2 e 3.

Portanto todas as farmácias foram aprovadas no teste de desintegração.

IV. Conclusão

A partir dos resultados obtidos no presente estudo, é possível concluir que as farmácias 1, 2 e 3 foram aprovadas em todos os testes a que foram submetidas. Sendo aprovadas nos testes de peso médio e desintegração, nota-se que as farmácias estão seguindo o mínimo preconizado pelas Boas Práticas de Manipulação de Medicamentos.

Por conseguinte, a determinação de peso médio e o testede desintegração permitem avaliar a qualidade de cápsulas manipuladas,assegurando a eficácia, rapidez e fácil execução no processo. No entanto, para se ter uma confiabilidade ainda maior, aconselha-se que seja feito outros ensaios como: teste de teor de fármaco, dissolução, pureza microbiológica.

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Antimicrobial Effect of Monovalent Copper Ions, Room Atmosphere Applications

By Magal Saphier, Bar Sabg, Gal Shraga, Semion Entus, Victor Chirovov, Stanislav Popov & Oshra Saphier

Abstract- This study continues the series of experiments revealing high antibacterial properties of monovalent copper ions (Cu^{+}). While previous studies showing that monovalent copper ions (Cu^{+}) are a robust antibacterial substance were conducted in an anaerobic atmosphere with acetonitrile as a ligand stabilizing monovalent copper ions [1,2], this study focuses on preparations that generated an effective antibacterial concentration of monovalent copper ions at room conditions.

We found that in a semi-hydrophobic environment, divalent copper with ascorbic acid (or a derivative of ascorbic acid) produces and maintains a stable concentration of monovalent copper ions [3].

Keywords: antibacterial effect; anti fungi, monovalent copper ions; e.coli.

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Antimicrobial Effect of Monovalent Copper Ions, Room Atmosphere Applications

Magal Saphier [α], Bar Sabg [σ], Gal Shraga [ρ], Semion Entus [ω], Victor Chirovov [¥], Stanislav Popov [§] & Oshra Saphier [χ]

Abstract- This study continues the series of experiments revealing high antibacterial properties of monovalent copper ions (Cu^{+}). While previous studies showing that monovalent copper ions (Cu^{+}) are a robust antibacterial substance were conducted in an anaerobic atmosphere with acetonitrile as a ligand stabilizing monovalent copper ions [1,2], this study focuses on preparations that generated an effective antibacterial concentration of monovalent copper ions at room conditions.

We found that in a semi-hydrophobic environment, divalent copper with ascorbic acid (or a derivative of ascorbic acid) produces and maintains a stable concentration of monovalent copper ions [3].

Moreover, we found that controlled diffusion of monovalent copper ions into the environment is inducing with the addition of surfactants.

The current study focuses on finding formulation generating monovalent copper ions in an aerobic atmosphere in sufficient concentration to disinfect contaminated solutions, surfaces, skin, west water, and more.

One of the developments is based on an ointment (Vaseline base). The ointments maintain an effective dynamic concentration of monovalent copper, the monovalent copper is obtained from the recycling redaction of divalent copper ions by ascorbic acid within the ointment.

The ointments were tested in vitro using the "Antimicrobial disk-diffusion susceptibility test", the results show the antibacterial efficacy of ointments samples on various bacteria, gram-negative and positive spores, and fungi like yeast.

This study presents a method for water disinfection based on formula absorbed on a sponge. The absorbed formula was submerged in the contaminated water. In this method, 0.025 g of formula eliminated 10^{4}/ml *E. Coli* bacteria from a liter of contaminated water.

Keywords: antibacterial effect; anti fungi, monovalent copper ions; e.coli.

I. Introduction

Recently published studies have demonstrated the antibacterial properties of monovalent copper ions, suggesting their robust activity in orders of magnitudes compared to silver ion (Ag^{+}) [1]. Divalent copper (Cu^{+2}) and metallic copper show no activity in controlled conditions [2]. The Cu^{+} ion antibacterial activity is intensified by high temperature, low molecular oxygen concentration, low pH, and poor carbon source [2]. On a minute time scale, Cu^{+} ion disinfected bacterial contamination [2]. Recently, patent applications demonstrate semi-hydrophobic ointments generating monovalent copper ions in an aerobic atmosphere in sufficient concentration to disinfect contaminated surfaces [3]. According to a recent study, Cu^{+} ions inhibit essential enzymes like DNA/RNA polymerase; it seems that the antibacterial mechanism is via enzymatic inhibition [4]. Copper's antimicrobial activities have been well recognized and exploited since ancient times for medicinal purposes [5]. The interest in antimicrobial applications of copper only increases with time. Currently, copper is widely used as a water purifier, fungicide, and bactericide. Ideas of introducing copper into cotton fibers, polymeric materials, and clothing to provide them biocidal properties were suggested more than a decade ago [6,7,8], and miscellaneous products are on the market already. Copper applications in healthcare might aid in successfully fighting bacterial contamination on solid surfaces and avoiding the spread of multidrug-resistant bacteria in hospitals [9]. All this makes understanding the processes resulting in the potent antibacterial effect of copper highly relevant.

Copper is an essential intracellular element in trace concentrations, while its excess causes toxicity [10]. Due to the ability of copper to exist in metallic and ionic forms, alternating between cuprous (Cu^{+}) and cupric (Cu^{2+}) oxidation states, its action on a variety of microorganisms, from fungi to bacteria, is a subject of ongoing research; most of the mechanisms and intracellular targets of this action are not yet elucidated [11]. For instance, in the case of yeasts, metallic copper surfaces mediated toxicity targets membranes, causing extensive membrane and envelope damage while not affecting DNA; this mechanism is known as a contact-mediated killing[12]. In the case of gram-positive bacteria, understanding molecular mechanisms leading to cell death caused by contact with both moist and dry copper surfaces is a controversial topic. It is reported that exposure of Staphylococcus Aureus to copper causes cell death through DNA damage [13]. In addition, cellular respiration is compromised, with little effect on cell membrane integrity [14].

In contrast, other studies exploring the toxic effect of copper surface contact with Staphylococcus haemolyticus cells suspension point at depolarization of

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the cytoplasmic membrane as the primary target and suggest that DNA degradation occurs only after cell death [15]. Regarding gram-negative bacteria, it is also shown that E.coli is rapidly killed on copper alloys surfaces [16]. The current model of a contact killing on dry surfaces characterizes this process as a cascade of events, such as successive cell membrane rupture and loss of cell content, copper ions influx into the cells leading to oxidative damage and DNA degradation, while the sequence of these events may differ [17]. In vivo, however, according to the literature, copper ions do not catalyze the formation of oxidative DNA damage [18,19,20]. Copper ions use as a weapon in the antimicrobial arsenal of grazing protozoa and phagocytic cells of the immune and affect central carbon metabolism in Staphylococcus aureus [21,22], which implies intracellular activity. At the same time, in our view, the role of dissolved mono copper ions that penetrate the cell through cation channels and paralyzes essential enzymes in the killing process should not be underestimated[4].

In aqueous solution, the common oxidation state of copper ions is bivalent (Cu^{2+}, cupric). Copper in the monovalent state (Cu^{+}, cuprous) remains in a low concentration due to a disproportion reaction (self-oxidation of monovalent copper to divalent copper and metallic copper), and due torapidlyoxidizedby molecular oxygento divalent copper.

Nevertheless, it is possible to elevate Cu^{+} ions concentration; adding reagents that form a more stable complex with Cu^{+} ions than with Cu^{2+} ions may achieve a high concentration of Cu^{+} ions in a deaerated aqueous environment. Acetonitrile [23, 24], benzoic acid [25] and ATP [26. 27] are good examples for Cu^{+} stabilizing reagents that shift the existing equilibrium between oxidation states to the formation of two Cu^{+} ions from one Cu^{2+} ion and metallic copper.

Our previous research [1,2] succeeded in exploiting this technique of Cu^{+} ions production, thus opening a series of studies devoted to an investigation of the antimicrobial effect of monovalent copper. We have clearly shown the superior efficacy of Cu^{+} ions over Cu^{2+} ions in killing *E. coli* and *Staphylococcus aureus* bacteria. Moreover, our studies have revealed that Cu^{+} ions had substantially higher efficacy than Ag^{+} ions, which are currently widely used as an antibacterial agent [1]. On the whole, our findings suggest that Cu^{+} should be considered as a potent antimicrobial agent.

II. Materials and Methods

a) Culture Media [28]

E. coli (NCIMB, str. K-12 substrate. MG1655) was stored in vials with 50% glycerol at -80oC until use. The strain was grown either in Luria broth medium (LB broth and agar, Difco). *E.coli* was grown in LB broth, typically containing 0.5% yeast extract, 1% bactotryptone and 1% NaCl. *S.A. Staphylococcus aureus, B.T. Bacillus thuringiensis, E.A. Enterobacter aerogenes, M.L. Micrococcus luteus, S.E. Staphylococcus epidermidis, S.F. Streptococcus faecalis, P.A. Pseudomonas aeruginosa, Delf Delftia tsuruhatensis. S.C. Staphylococcus cohnii, B.B. Brevibacillus brevis, and beer yeast* was grown and treated according to the appropriate procedure that appears in the literature.

b) Preparation of Starter and Growth Methods [28]

i. *Preparation of starter and bacterial growth in LB broth medium*

LB broth was inoculated by an *bacteria* colony grown on LB agar. The starter was grown overnight in a rotary shaking incubator (37 °C, 170 rpm). The next day, the starter was seeded into fresh LB media at 1:100 dilution and grown to OD600 0.3-0.4 for 2-3 hours to bring the bacteria to the exponential growth phase. The resulting bacterial suspension was finally inoculated into fresh LB at 1:100 dilution.

ii. *Preparation of ointments*

To make the ointment, heat Vaseline on a water bath until melting, to which add while stirring all the ingredients except ascorbic acid or its Palmitate derivative. The mixture cooled while stirring, and only then, the ascorbic acid was added while stirring, obtaining a homogeneous ointment.

c) Antimicrobial disk-diffusion susceptibility test [29]

With a piece of a wadded disk having 3mm diameter, an amount of 0.1 gr' ointment was taken, ensuring ~1 mm thickness layer, and was put in the center of inoculated LB agar in a Petri dish. The test ointment samples and control (inoculated the same way but having no ointment) in Petri dishes were put into the incubator for 18 hours at 37 °C. Each ointment composition was done in a triplicate for standard deviation calculation. Measurements of a zone without bacterial growth, e.g., distance between the edges of ointment and bacterial growth areas, were performed using a ruler.

i. *Counting with colony-forming units*

For estimates, the number of bacteria or fungal cells in a sample, we used the colony-forming units (CFU) counts. Bacteria were counted using a routine CFU technique, i.e., plating bacteria from serial dilutions onto LB agar and incubating overnight at 37 °C.

d) Stability test of formulations [30]

To check the stability of the formulations, for hot storage conditions, was 37 °C incubator was used, and for room storage conditions, samples were stored in the lab. The storage conditions in a closed container were tested and exposed to air conditioning Petri dishes. Before and after storage, each ointment sample was

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tested for antimicrobial activity by measuring bacterial growth inhibition on triplicated Petri dishes on LB agar.

i. *Cooper ions Diffusion test*

Eight Petri dishes were prepared for the experiment: four were sterile, and the others were seeded with bacterial inoculation. The bacteria-containing plates were used to determine the bacterial influence on copper diffusion. The ointment was prepared and placed on the center of each dish, and the Petri dishes were placed into the incubator at 37 °C for the defined time intervals: 1, 2, 3, or 4 hours. After that, each set, consisting of bacteria containing and sterile dish withdrawn from the incubator and four small rings of agar cut off. Each agar sample dissolved with hot nitric acid, and the ICP-OES technique used to measure the copper concentration. The map of a Petri dish used for cutting off agar samples is displayed in Fig. 10, with inner-outer radiuses measured starting from the edge of the ointment sample.

III. Results and Discussion

a) Antibacterial activity of ointments

Several antibacterial compositions developed, creating a durable and effective concentration of monovalent copper ions.

Table 1: Shows the compositions that we will refer to in the results.

	Amount (%w/w)			
Material\ Composition name	A Emulsifying base	B Absorption base	C Absorption base+ S.A	D Encourages diffusion
Copper(II) gluconate	10.0	10.0	10.0	0
Copper sulfate	0	0	0	3.2
Ascorbyl Palmitate	6.5	6.5	7.0	0
Ascorbic Acid	0	0	0	4.0
Vaseline	20.0	27.0	42.0	27.0
Glycerol	10.7	22.5	14.0	27.0
Water	30.0	5.0	2.0	1.8
Stearyl alcohol	20.0	0	0	0
Lanoline	0	27.0	0	0
Isopropyl Myristate	0	0	8.0	27.0
Salicylic acid	0	0	17.0	0
Sodium lauryl sulfate	0.8	0	0	11.0
Copper dust	2.00	2.00	0	0
Acetonitrile	0	0	0	0

Figure 6 displays the inhibition radius of bacterial growth caused by different copper (II) gluconate concentrations for emulsion (A) and absorption (B) bases ointments, respectively.

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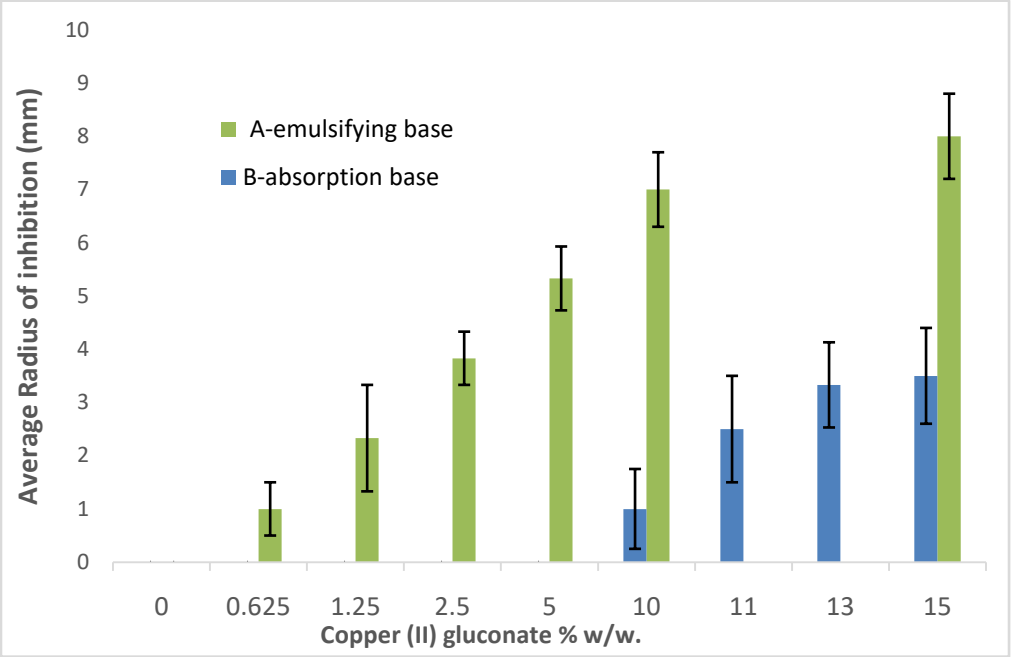


Figure 1: Impact of copper (II) gluconate mass concentration for emulsifying (A)and absorption (B) base ointments (The compositions are listed in the table 1) on *E. Coli* bacterial growth inhibition radius.

Figure 6 points to the solid correlation of copper (II) gluconate concentration in the ointments (A and B) and its antibacterial effect. That meets our expectations since the Cu^{+} ions generation mechanism strongly depends on reactants' concentrations: the more Cu^{+2} ions are involved in reactions, the more Cu^{+} ions are generated and could be diffused throughout agar and create the larger bacterial-free region.

Figure 7 presents the results of *E. Coli* bacterial growth inhibition radius for ointments A (Cu^{+}), ointments A with copper (II) gluconate but without the reduction elements (Ascorbyl Palmitate and Copper dust) (Cu^{+2}) that show limited activity, and (Ag^{+}) resulting from replacing the copper ions in the same molar concentration with silver ions (Ag^{+}). The last show limited activity as well.

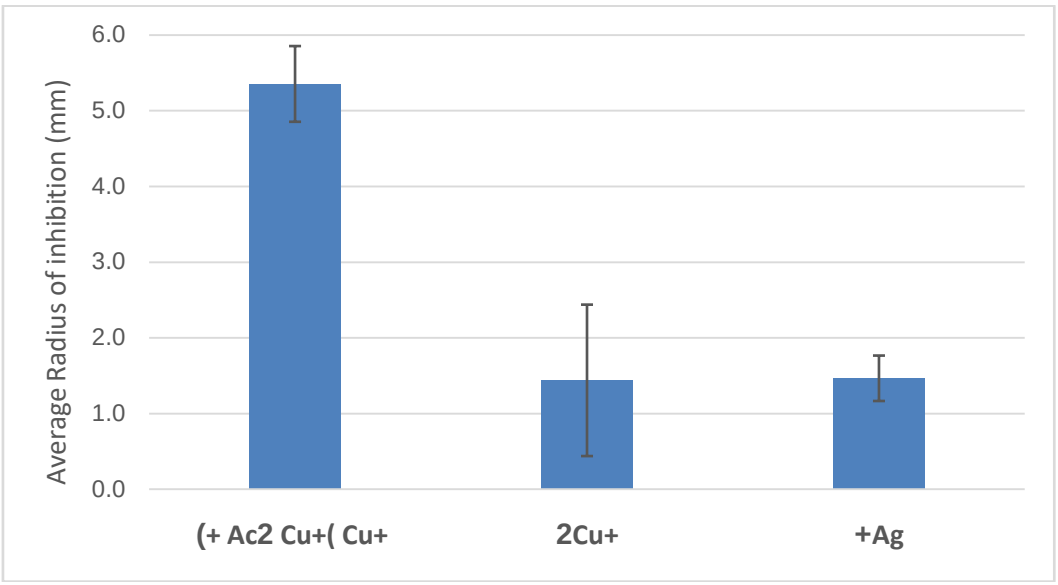


Figure 7: Impact of ions nature (Cu^{+}. Cu^{+2} and Ag^{+}) in emulsifying (A) ointments on *E. Coli* bacterial growth inhibition radius

Figure 7 shows that the effect of monovalent copper ions is much more significant than monovalent silver ions used commercially to control bacteria growth. Divalent copper ions have a particular effect attributing to a small concentration of monovalent copper ions obtained from divalent copper ions depending on the nature of the redox potential of the environment.

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Comparison of the effect of type A ointment on *E. Coli*, *Bacillus thuringiensis* (*B.T*) bacteria, and *beer Yeast* on the growth inhibition radius presented in figure 8.

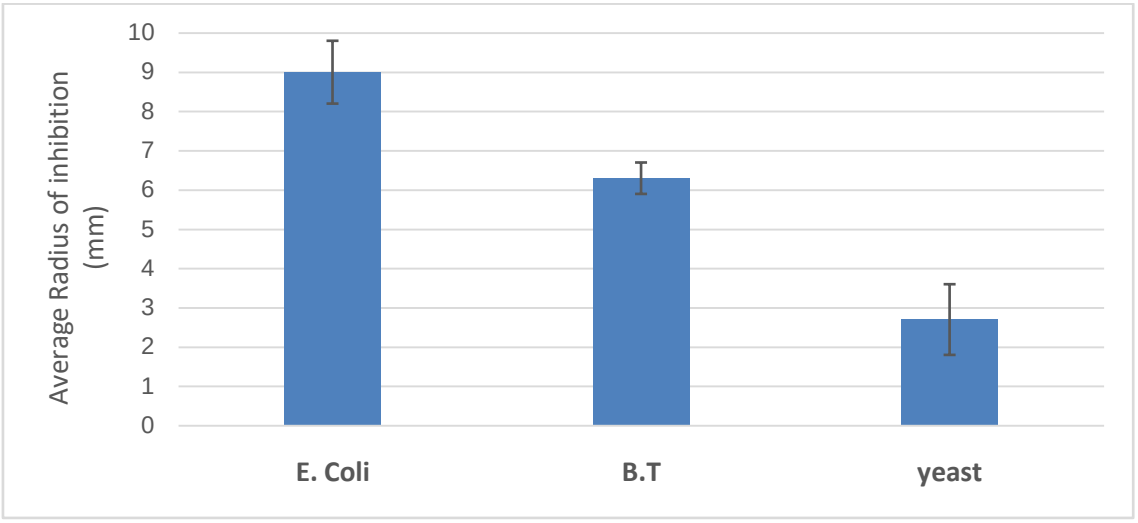


Figure 8: Comparison of the effect of type A ointment on *E. Coli*, *Bacillus thuringiensis* (*B.T*) bacteria, and *beer Yeast* on growth inhibition radius.

The results show that gram-negative bacteria (represented by Figure 8 by *E. Coli*) are more sensitive than gram-positive bacteria (represented by Figure 8 by *Bacillus thuringiensis (B.T)*) and fungi (represented by Figure 8 by *beer Yeast*) to monovalent copper ions. The ointments (A, B, C) were successfully tested on about 12 types of bacteria: *E.C. Escherichia coli, S.A. Staphylococcus aureus, B.T. Bacillus thuringiensis, E.A. Enterobacter aerogenes, M.L. Micrococcus luteus, S.E. Staphylococcus epidermidis, S.F. Streptococcus faecalis, P.A. Pseudomonas aeruginosa, Delf Delftia tsuruhatensis. S.C. Staphylococcus cohnii, B.B. Brevibacillus brevis.*

In an attempt to increase the inhibitory capacity of the formula without metallic copper, salicylic acid is added to the formula (ointments C). The choice in Salicylic acid is due to studies indicating that aromatic compounds stabilize monovalent copper [25] and because salicylic acid is FDA approved and is widely used in the cosmetics industry. Figure 9 displays the inhibition radius of bacterial growth caused by different concentrations of salicylic acid in ointments C composition.

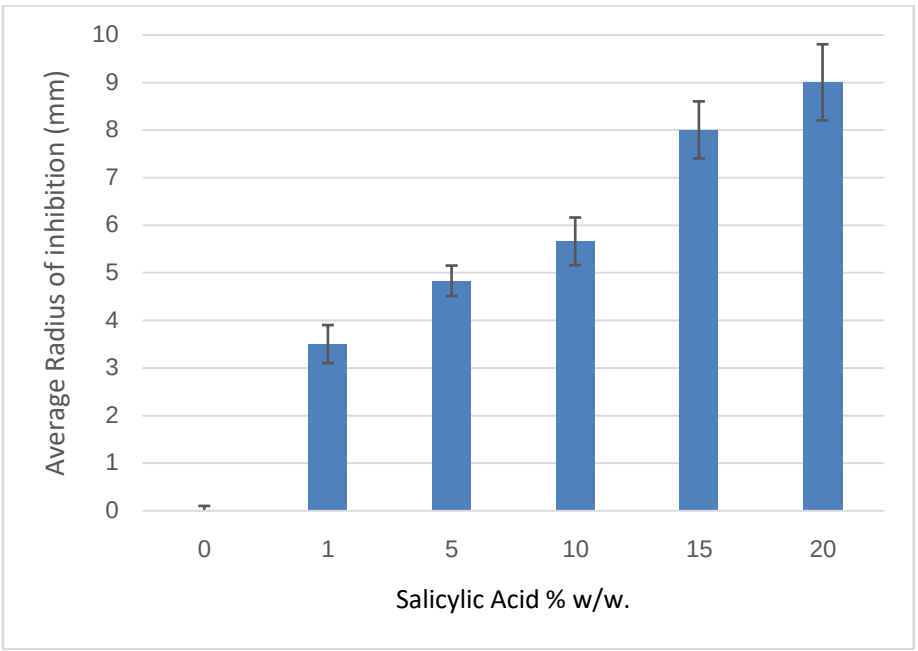


Figure 9: The relation between inhibition radius of *E. Coli* bacterial growth and salicylic acid mass concentration for absorption base ointment (C), control using salicylic acid without the copper ion, give no inhibition effect.

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Salicylic acid forms a stabilizing complex with monovalent copper, the stability constant (~1000M^{-1}) published in the literature [25]. The results shown in Figure 9 correspond to the above stabilization. Adding a stabilizing agent to monovalent copper ions compensates for the lack of metallic copper that serves as a reducing reservoir.

To test the stability of the ointments (A and B), they were kept for six months at room temperature, and 37 ° C. Figure 10 presents the results.

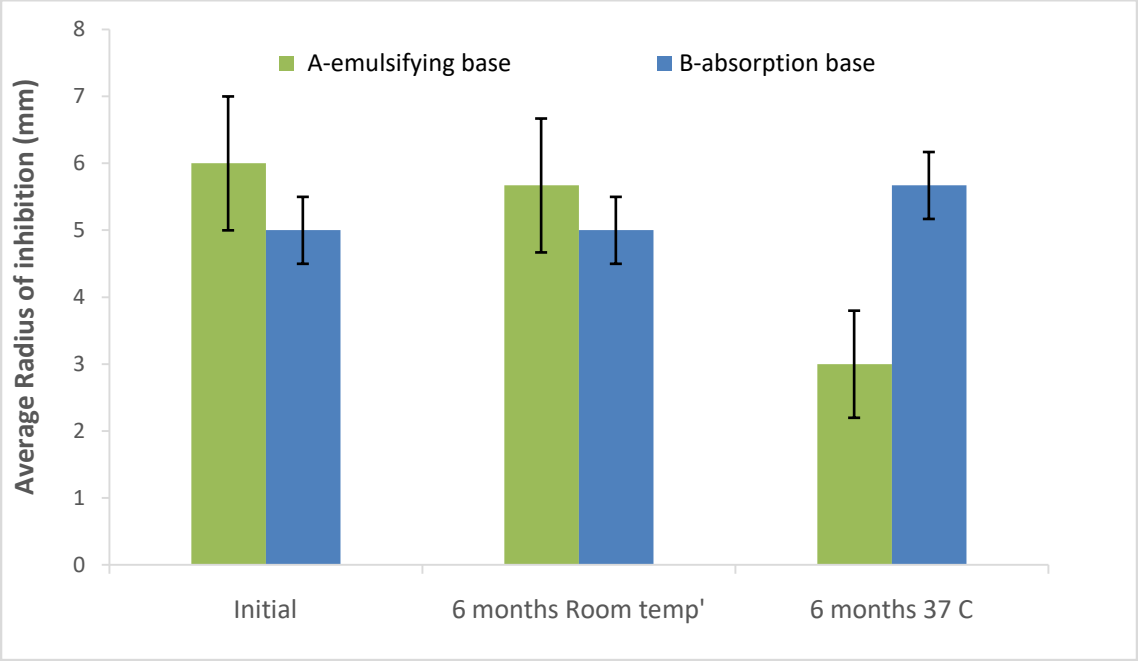
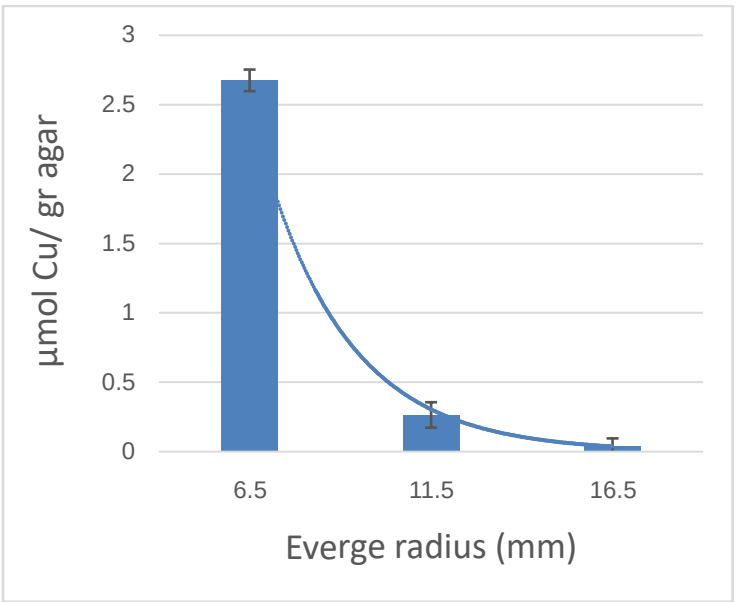


Figure 10: Impact of storage time (stability test) on *E. Coli* bacterial growth inhibition radius.

Figure 10 shows that while the effectiveness of type A ointment is deteriorating over time, the effectiveness of type B ointment is maintained and increases over time.

b) Diffusion of Cu(I) ions through agar

Figure 10 displays the concentration of Cu on the agar taken from the LB agar Petri dish. According to the diagram in Figure 10 left, the agar cut into rings, the ring dissolved in nitric acid, and the copper ions were determined using ICP technology. The experiment was performed parallel on a sterile agar and a bacterium-seeded agar.



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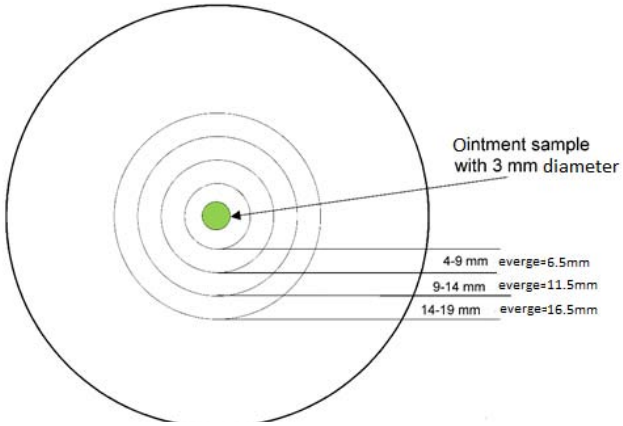


Figure 10: The change in the copper concentration in LB agar (3 hours from the application at 37 °C) ointment type A sample (left) and the map of a Petri dish used for cutting off agar samples.

Figure 10 (left) illustrates the diffusion of copper ions into the agar. As expected, there is an exponential dependence on the radius. A good match was obtained between the diffusion of the copper ions, and the radius of bacterial inhibition, the bacterial inhibition radius in the same condition was 9mm. It is possible to conclude that the ~0.5 μmol Cu^{+}/gr agar concentration is lethal to *E. coli* bacteria.

c) *Water disinfection based on formula D absorbed on sponge*

Figure 11 shows the ability to disinfect water from *E.Coli* bacteria with the help of a type D ointment. The ointment was absorbed into a medical sponge in which it was in contact with the contaminated water.

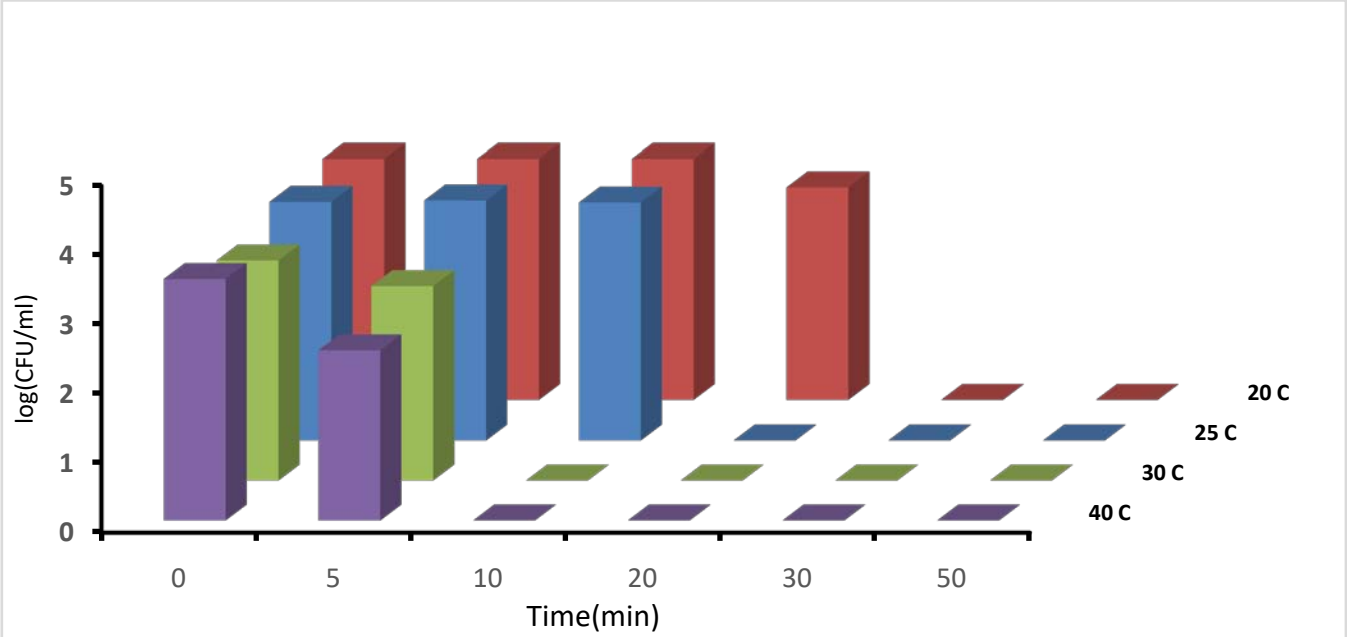


Figure 11: The relation between CFU of *E. Coli* bacteria in liter solution and time from submerging 0.025g ointment (D) absorb on a sponge in different temperatures

The composition of type D ointment allows the diffusion of monovalent copper ions into the water. The concentrations are sufficient for disinfection (0.2ppm) but below the permitted concentration of copper ions in drinking water (1.3ppm). The temperature has a dramatic effect. Below 25°C, the efficiency drops, and it takes much longer for complete disinfection of the solution. These results are consistent with previous research showing a dramatic effect of temperature on the antibacterial activity of monovalent copper ions [2].

Table 2 indicates the accepted values for water testing and the values measured to the treated water display in fig 11.

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Table 2

Temp (°C)	Cu ions (ppm)	TN (ppm)	TOC (ppm)	Conductivity (μs/cm)	pH
20	0.20±0.05	0.54±0.01	6.30±0.19	283±3.54	7.62±0.01
25		0.43±0.02	6.10±0.01	290±7.78	8.04±0.08
30		0.29±0.03	6.48±1.04	253±3.54	8.40±0.01
The standard for drinking water [31,32]	1.3 (USA) 2 (Europe)	10	25	500-1000	7.5-8.5

The water obtained by this method meets the standard; even the amount of organic carbon (TOC) is below the allowed value.

IV. Conclusions

This study demonstrates how to utilize the understanding that monovalent copper ions are the active form in the antibacterial capacity of copper and its practical developments in room conditions.

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Solvent Polarity and Temperature Effects on Extracted Secondary Metabolite from the Fruit of *Tetrapleura Tetraptera* and its Antibacterial Potential on Uropathogens

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Abstract- Odoriferous medicinal plants are known to be used as natural therapeutic agents, as they are rich sources of terpenoids and polyphenols. This study was aimed at evaluating the solvent polarity, temperature effects and synergistic potential of the phytochemicals in the fruit extract obtained from the fruit of *Tetrapleura tetraptera* on clinically isolated uropathogens. *T. tetraptera* has being used locally in treating some ailments. The sample was extracted using methanol, hot water and cold water respectively. The quantitative and qualitative compositional analysis of the secondary metabolites of the fruit extract was carried out using Gas chromatography-mass spectrometry (GC-MS). The antibacterial screening was carried out using agar-well diffusion assay. The GC-MS analysis of the fruit extract led to the identification of thirty-five (35) constituents amounting to 96.28% of the extract.

Keywords: tetrapleura tetraptera, fruit extract, phytochemical, pathogen, natural antibiotic.

GJMR-C Classification: DDC Code: 150.195 LCC Code: BF408



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Solvent Polarity and Temperature Effects on Extracted Secondary Metabolite from the Fruit of *Tetrapleura Tetraptera* and its Antibacterial Potential on Uropathogens

Alao, Felix O. [α], Ololade, Zacchaeus S. [σ] & Garba, Jamiu O [ρ]

Abstract- Odoriferous medicinal plants are known to be used as natural therapeutic agents, as they are rich sources of terpenoids and polyphenols. This study was aimed at evaluating the solvent polarity, temperature effects and synergistic potential of the phytochemicals in the fruit extract obtained from the fruit of *Tetrapleura tetraptera* on clinically isolated uropathogens. *T. tetraptera* has being used locally in treating some ailments. The sample was extracted using methanol, hot water and cold water respectively. The quantitative and qualitative compositional analysis of the secondary metabolites of the fruit extract was carried out using Gas chromatography-mass spectrometry (GC-MS). The antibacterial screening was carried out using agar-well diffusion assay. The GC-MS analysis of the fruit extract led to the identification of thirty-five (35) constituents amounting to 96.28% of the extract. Alletone (16.9%), 3-hydroxydihydro-2(3H)-furanone (10.0%), 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (9.3%), 2,3-dihydothiophene (4.5%), 5-hydrxoymethylfurfural (9.0%) and (4E)-4-methyl-4-hepten-3-one (9.0%) were the most abundant components in the fruit extract. These secondary metabolites greatly showcased the antimicrobial potential of the fruit of *T. tetraptera.* The findings of this study showed that there was inhibitory effect of *T. tetraptera* extracts on all the tested organisms. The sample exhibited antibacterial properties against Gram positive and Gram negative organisms with the methanol extract showing the highest inhibitory effect. Hot water and cold water showed similar inhibitory effects. The zones of inhibition ranged from 8-21 mm. This study affirms the traditional application of the sample since it revealed that it possesses antimicrobial properties which can be used for the treatment of a wide range of diseases.

Keywords: tetrapleura tetraptera, fruit extract, phytochemical, pathogen, natural antibiotic.

I. Introduction

Medicinal plants have been an important source of natural drugs and play essential role in healthcare. A wide range of medicinal plants used as traditional medicine have been found to cure various human diseases, which are associated with microbial infections (Dar *et al.,* 2017; Oyedemi *et al.,* 2018; Ololade and Anuoluwa, 2020; Ugboko *et al.,* 2020). Phytochemicals have therefore provided the best alternative method for disease treatment and management (Oyelese *et al.,* 2020). It was discovered long ago that some plant materials exhibit antibacterial properties. Recently, there is a growing demand globally by consumers in minimizing artificial preservation that can be detrimental to human health. Consequently, spices, herbs and naturally occurring phenolics from various plants sources are being studied in detail in response to consumer requirements for fresher and more natural additive-free products (Asif, 2015; Lourenco *et al*., 2019; Ulewicz-Magulska and Wesolowski, 2019; Adesina *et al*., 2021). Plants derives medicines are of immense benefits since they are relatively safer than synthetic alternatives, they offer profound therapeutic benefits and are more affordable source of treatment (Atanasov *et al*., 2015; Anand *et al*., 2019; Ololade *et al*., 2021). Plant based antimicrobials therefore represent a vast untapped source of medicines.

Tetrapleura tetraptera (Schumach. and Thonn Taub) is from the family of Mimosaceae and commonly known as "Aridan" in Nigeria. The medicinal plant is a perennial tree with dark green leaves and thick, woody base and spreading branches. The fruit consist of a fleshy pulp with small, brownish-black seed. The fruit possess a fragrant characteristic pungent, aromatic odour and flavour which has been attributed to insect repellent property (Odesanmi *et al*., 2010; Atawodi *et al*., 2014; Nwoba, 2015; Ozaslan *et al*., 2016; Larbie *et al*., 2020; Otimanam *et al*., 2020). Medicinally, the fruit is used to prepare soup or porridge for nursing mothers from the beginning of childbirth to prevent post-partum contraction, gastro-intestinal disorders especially stomach ulceration and to aid lactation in nursing mothers (Mpody *et al*., 2019). It has also been harnessed in the management of convulsions, leprosy, inflammation, flatulence, jaundice, malaria, rheumatism onset of diabetes mellitus in adults and as a molluscide (Uyoh *et al*, 2013).

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II. Materials and Methods

a) Collection of plant material

The fruit samples were randomly obtained from Ota, Nigeria and identified by botanists as *Tetrapleura tetraptera* in the Department of Biological Science, Bells University of Technology, Ota, Ogun State, Nigeria.

b) Sample Preparation and Extraction

The fresh fruit pods of *T. tetraptera* were air dried and stored in air tight containers until required for use. The pods were cut into small sized pieces before pulverization using laboratory mortar and pestle and finally into powder with an electric blender. Pulverised sample was weighed with an analytical balance, 30 g were soaked in methanol, hot water and cold water respectively for three days with intermittent shaking. The extracts solutions were filtered and then concentrated using water bath (Ololade and Abiose, 2019).

c) GC-MS Phytochemical Screening of the Fruit Extract of T. tetraptera

The methanolic extract of *T. tetraptera* fruit was analysed using Shimadzu GC-MS-QP2010 Plus (Japan). The separations were carried out using a Restek Rtx-5MS fused silica capillary column (5%-diphenyl-95%-dimethylpolysiloxane) of 30 m× 0.25 mm internal diameter (di) and 0.25 mm in film thickness. The conditions for analysis were set as follows; column oven temperature was programmed from 60-280°C (temperature at 60°C was held for 1.0 min, raised to 180 °C for 3 min and then finally to 280 °C held for 2 min); injection mode, Split ratio 41.6; injection temperature, 250 °C; flow control mode, linear velocity (36.2 cm/sec); purge flow 3.0 ml/min; pressure, 56.2 kPa; helium was the carrier gas with total flow rate 45.0 ml/min; column flow rate, 0.99 ml/min; ion source temperature, 200 °C; interface temperature, 250 °C; solvent cut time, 3.0 min; start time 3.5 min; end time, 24.0 min; start m/z, 50 and end m/z, 700. Detector was operated in EI ionization mode of 70 eV. Components were identified by matching their mass spectra with those of the spectrometer data base using the NIST computer data bank, as well as by comparison of the fragmentation pattern with those reported in the literature.

d) Preparation of Extract Solution for Antimicrobial Test

Stock solutions of the concentrated (methanol, hot and cold) fruit extracts (2.5mg/ml, 2.0mg/ml, 1.5mg/ml, 1.0mg/ml, and 0.5mg/ml) were prepared in dimethyl sulfoxide (DMSO). The solutions were stored in the refrigerator until time for use (Alao *et al*., 2018).

e) Antimicrobial Assay

Collection of isolates: Uropathogenic organisms which were identified as Staphylococcus aureus, Staphylococcus saprophyticus, Escherichia coli, Enterococcus faecalis and Pseudomonas aeruginosa were obtained from the stock collection of the Microbiology Laboratory of Bells University of Technology Ota, Nigeria. Stock solutions of the concentrated (methanol, hot and cold) fruit extracts (2.5mg/ml, 2.0mg/ml, 1.5mg/ml, 1.0mg/ml, and 0.5mg/ml) were prepared in dimethyl sulfoxide (DMSO). The solutions were stored in the refrigerator until time for use (Alao *et al*., 2018). In vitro antibacterial potential of the crude extracts were evaluated using agar well diffusion method.

Antibiotic Susceptibility Test: Antibiotic susceptibility test was carried out on each of the pathogenic isolates to determine their susceptibility to the conventional antibiotic dics. Multi-sensitivity discs bearing eight different antibiotics Augmentin, Ceftazidime, Cefuroxime, Cotrimoxazole, Cloxacillin, Erythromycin, Gentamicin, and Ofloxacin were aseptically placed with the aid of sterile forceps on inoculated Mueller Hinton plates. The plates were incubated at 37°C for 24 hr (Hombach *et al*., 2015; Alao *et al*., 2018; Oka and Nweze, 2020).

III. Results and Discussion

a) Phytochemical Composition of the Fruit Extract of T. tetraptera

In this study, the fruit of *T. tetraptera* was investigated for its chemical constituents. The colours were dark green and brown, respectively. The concentrated extract was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis for detailed identification of its components. Identification of the compound was also aided by comparison of their GS-MS mass spectra database. The retention indices of each identified components were also calculated based on their retention time in order to confirm the identification. The GC-MS analysis of the fruit extract of *T. tetraptera* led to the identification of 35 constituents representing 96.28% of the extract. The compound, retention indices and percentage composition are given in Table 1, where the identified components were listed in order of their retention indices. The GC-MS analysis of the fruit extract of *T. tetraptera* led to the identification of 35 constituents representing 96.28% of the extract. Alletone (16.9%), 3-hydroxydihydro-2(3H)-furanone (10.0%), 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (9.3%), 5-Hydrxoymethylfurfural (9.0%), (4E)-4-methyl-4-hepten-3-one (9.0%) and 2,3-dihydothiophene (4.5%) were the most abundant components in the fruit extract of *T. tetraptera*. These compounds contribute greatly to the antimicrobial effects of *T. tetraptera*. The above results showed that the fruit extract of the sample grown in Nigeria and other West African countries has various medicinally active compounds and properties that have been used to treat a great variety of human diseases such as convulsions, leprosy, inflammation, flatulence, jaundice, malaria, adult onset of diabetes mellitus,

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rheumatism and as a molluscide. The findings of this study showed that *T. tetraptera* can be used in developing antibacterial substances in combating multidrug resistant bacteria.

Table 1: Chemical Composition of the Fruit Extract of *Tetrapleura tetraptera*

Compound	Retention Index	% Composition
3-methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene	0	0.6
3-methyl-2-heptanol	130	1.6
sec-butyl nitrite	544	0.2
tutane	598	1.0
sec-butylamine	598	1.0
N-methylisobutylamine	653	1.0
2,3-dihydothiophene	723	4.5
3-methyl-3-ethylpentane	732	2.5
N-methyl-N-(4-pentenyl)amine	806	1.0
propylene Carbonate	875	0.2
pimelic ketone	891	6.3
(4E)-4-methyl-4-hepten-3-one	938	9.0
3-hydroxydihydro-2(3H)-furanone	1013	10.0
alletone	1022	16.9
1,3-butylene glycol diacetate	1087	0.03
octylmegthylamine	1114	1.0
5-hydrxoymethylfurfural	1163	9.0
(+/-)-citronellol	1179	0.03
3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	1269	9.3
(2E)-2-undecenyl acetate	1489	0.03
decane-1, 10-diol	1501	13.0
1-ethyldecyl acetate	1516	0.03
D-glucitol, 1,4:3,6-dianhydro-, dinitrate	1678	0.2
myristic acid	1769	3.2
methyl 14-methylpentadecanoate	1814	0.1
palmitic acid, methyl ester	1878	0.1
methyl 15-methylhexadecanoate	1914	0.1
palmitic acid	1968	3.2
phytol	2045	0.03
trans-phytol	2045	0.03
methyl elaidate	2085	0.1
methyl (10E)-10-octadecanoate	2085	0.1
methyl cis-octadec-11-enoate	2085	0.1
linolelaidic acid, methyl ester	2093	0.6
1,4-diacetyl-3-acetoxymethyl-2,5-methylene-1-rhamnitol	2105	0.2
Percentage Total		**96.28**

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b) Antibacterial Screening of the Fruit Extract of T. tetraptera

In this study, different concentrations of the methanolic, hot water and cold water extracts of the fruit of *T. tetraptera* (2.5, 2.0, 1.5, 1.0, 0.5 mg/ml) were prepared) and tested on six pathogens (*Staphylococcus aureus, Staphylococcus saprophyticus, Enterococcus faecalis, Serratia marcescens, Proteus mirabilis and Pseudomonas aeruginosa*). Inhibition zones were observed for the tested organisms. The results obtained for each organism were shown in figure 1-6. Antibiotic sensitivity and resistance patterns of the isolates to standard antibiotic disc were shown in table 2. In this study, the leaves and fruit of this plant were used to determine the antimicrobial activity. The plant extracts were prepared using methanol, hot water and cold water by solvent extraction procedures and their antimicrobial properties were assessed using agar well diffusion method. The sample exhibited antibacterial properties against Gram positive and Gram negative organisms. The methanol extract showed the highest inhibitory effect. Then, hot water and cold water had similar inhibitory effects. The fruit had similar zone of inhibition ranging from 8-21 mm. However, fruit extract had wider range of activity at different concentrations. *P. aeruginosa* showed the highest zone of inhibition among the tested bacteria with the fruit extract was with a maximum zone of inhibition of 20 mm. For *Staphylococcus aureus*, the highest inhibitory effect was observed in methanol extract as depicted in figure 1. This ranged between 10-19 mm at various concentrations used in this study.

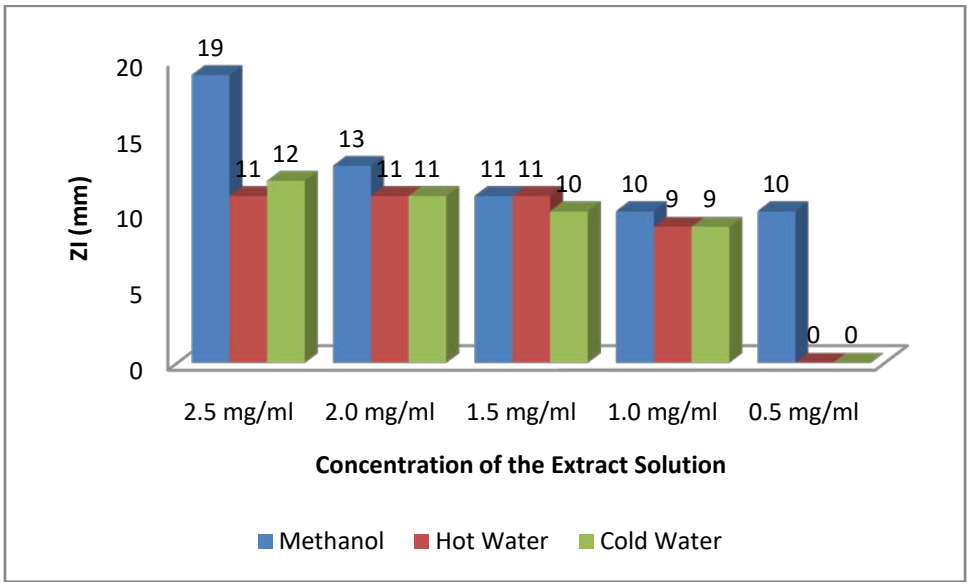


Figure 1: Antibacterial Activity of *T. tetraptera* Fruit Extract on Staphylococcus aureus

For *Staphylococcus saprophyticus,* the highest inhibitory effect was observed in hot water extract as shown in figure 2. This was ranged between 09-15 mm at various concentrations used in this study.

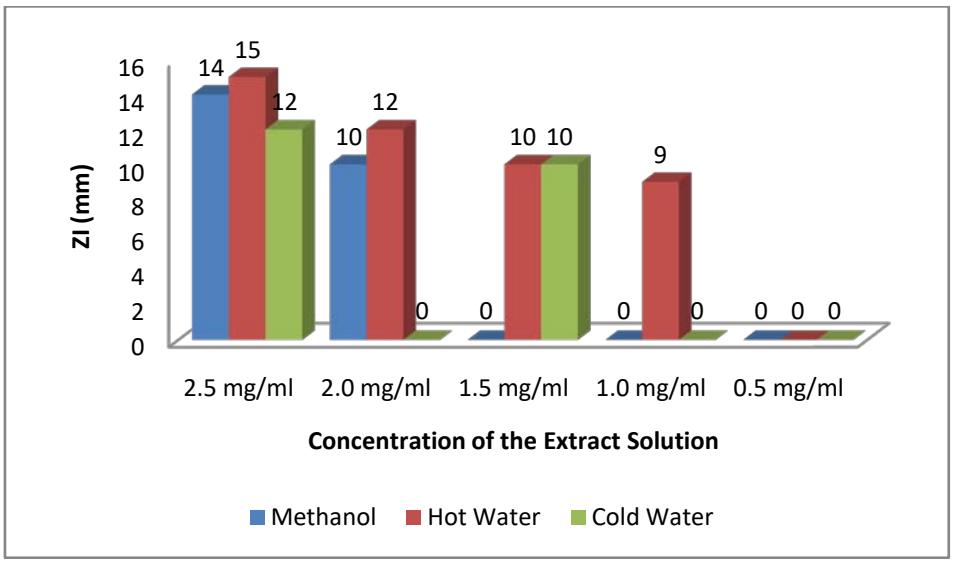


Figure 2: Antibacterial Activity of *T. tetraptera* Fruit Extract on Staphylococcus saprophyticus

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For *Enterococcus faecalis,* the highest inhibitory effect was observed in cold water extract, followed by hot water extract and least by the methanol extract as shown in figure 3. This was ranged between 08-21 mm at various concentrations used in this study.

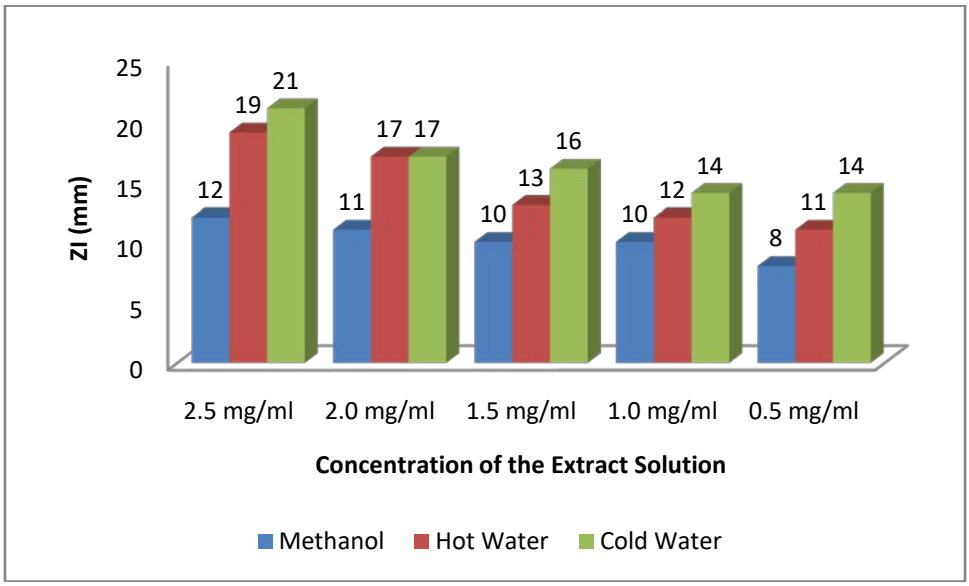


Figure 3: Antibacterial Activity of *T. tetraptera* Fruit Extracts on Enterococcus faecalis

For *Serratia marcescens,* the highest inhibitory effect was observed in methanol extract, followed by hot water extract and then cold water extract as shown in figure 4. The value of zones of inhibition was ranged between 09-21 mm at various concentrations considered in this study.

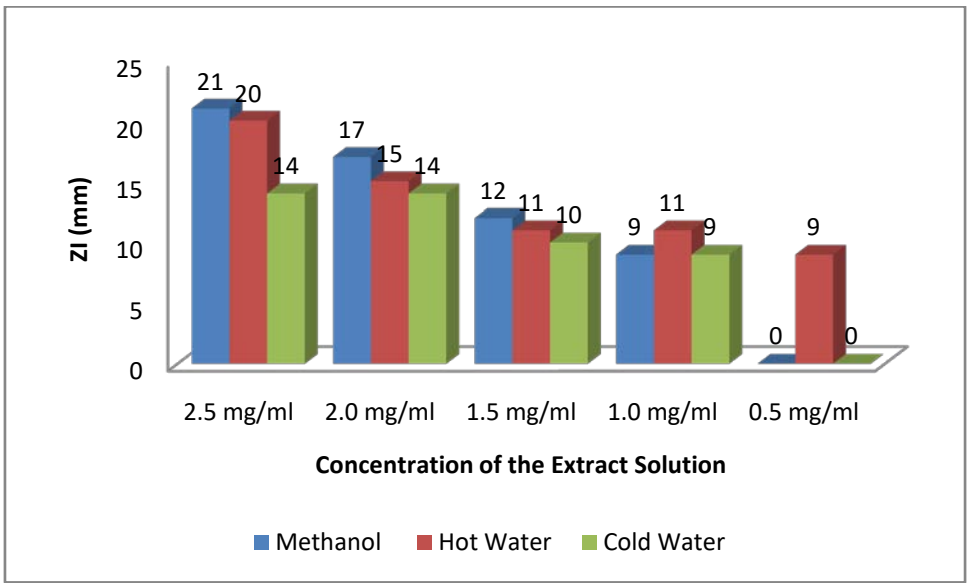


Figure 4: Antibacterial activity of *T. tetraptera* Fruit Extract on Serratia marcescens

For *Proteus mirabilis,* the highest inhibitory effect was observed in methanol extract and cold water extract and then hot water extract did not show activity except at 2.5 mg/ml as shown in figure 5. The value of zones of inhibition was ranged between 09-18 mm at various concentrations considered in this study.

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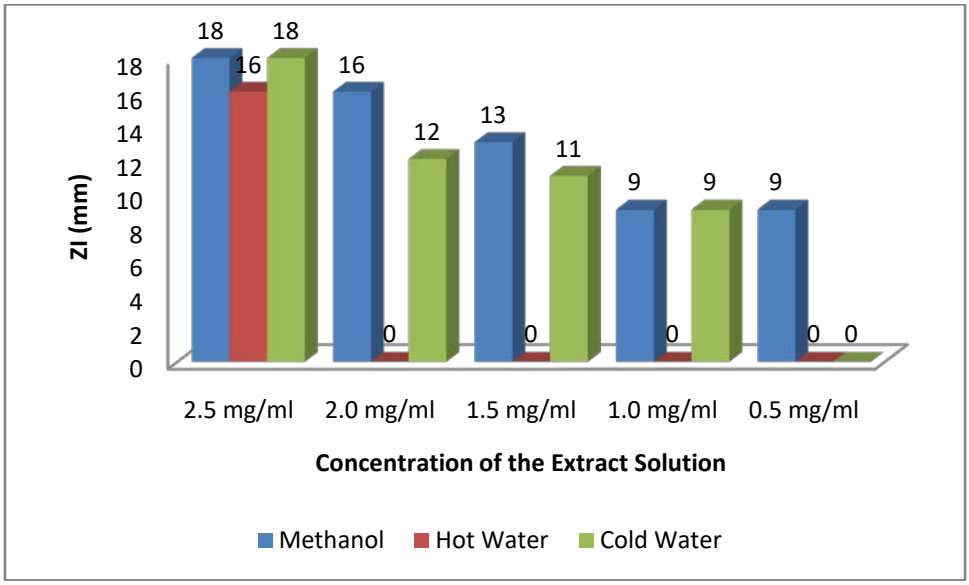


Figure 5: Antibacterial Activity of *T. tetraptera* Fruit Extracts on Proteus mirabilis

For *Pseudomonas aeruginosa,* the highest inhibitory effect was observed in methanol extract followed by hot water extract and then cold water extract as shown in figure 6. The value of zones of inhibition was ranged between 11-20 mm at various concentrations considered in this study.

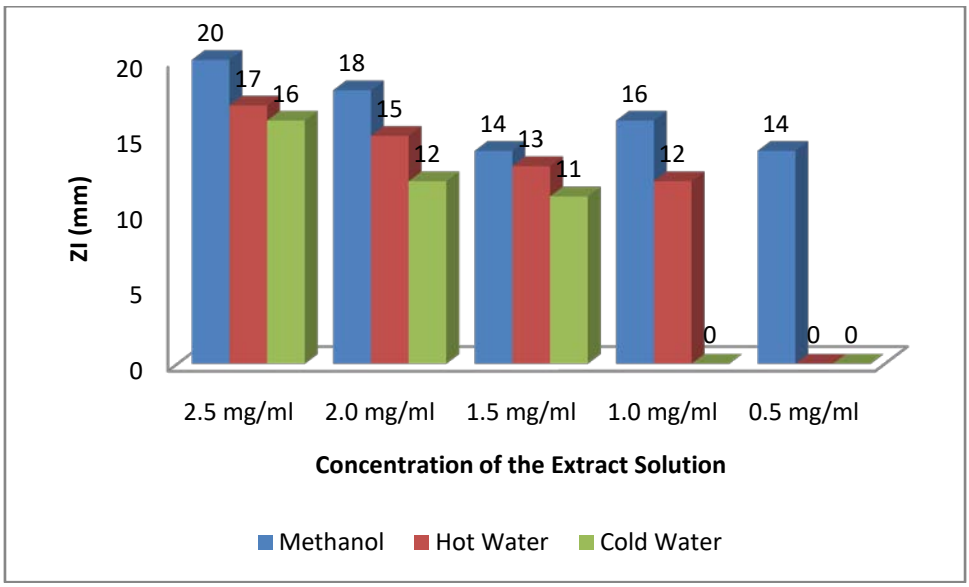


Figure 6: Antibacterial Activity of *T. tetraptera* Fruit Extracts on Pseudomonas aeruginosa

In addition, the effect observed was dependent on the concentration of the extracts and the extract established an interaction with the concentration used as the range of activity reduced with the decrease in concentration of each extraction solvent. Finally, the effects measured was also dependent on the extraction method and solvent (absolute methanol, hot water and cold water) used and the fruit established an interaction with the extraction method. Table 2 showed the susceptibility of the tested organisms to different antibiotics. All of them were inhibited by at least one antibiotic with no exception. They were all resistant to Augmentin, Ceftazidime, Cefuroxime, and Cloxacillin. Also, the findings from this study indicated higher resistance pattern exhibited by the organism to synthetic antibiotic in comparison to the high inhibitory effects of *T. tetraptera* extracts against these organism. Therefore, if the plant can be adequately harnessed and studied, it can be used as a natural antibacterial agent against some of the pathogens as discovered in this study.

Solvent polarities are factors that responsible for the variation in the antibacterial activity of plant extracts, permeability of cell of bacteria, concentration etc (Gonelimali *et al*., 2018; Zhang *et al*., 2020). The effect of solvent polarity on extraction yield and antibacterial properties of secondary metabolites in the fruit was studied. Solvent type and polarity index play an important role in the antibacterial activities level in the

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extracts (Truong *et al*., 2019; Wakeel *et al*., 2019). Extraction in highly polar solvents resulted in high extract yield of phytochemicals. The polarity-dependent increase in antibacterial potential indicates the extraction of strong antimicrobial compounds in polar solvents. The quantities of crude extracts with different solvents were different in different extracts reported that the extracts of these solvents have significantly different antimicrobial activity (Altemimi *et al*., 2017; Nawaz *et al*., 2019). The different antimicrobial activities of these solvents and plants parts might be because of the different types and quantity of biological compounds in these extracts. The role of solvent polarity in the quantity and quality of crude extracts, secondary metabolites, and biological activities cannot be over emphasized. Differences in the antibacterial activities of the may be because of the phytochemical polarity index and their association with solvent polarity index. Similar polarity index containing solvents can dissolve phytochemicals that have similar or close related polarity index (Othman *et al*., 2019; Chassagne *et al*., 2021; Vaou *et al*., 2021).

Table 2: Antibiotic Sensitivity and Resistance Patterns of Isolates

Isolates	OFL 5µg	AUG 30µg	CAZ 30µg	CRX 30µg	GEN 10µg	CTR 30µg	ERY 15µg	CXC 5µg
E. faecalis	34	R	R	R	21	12	R	R
P. aeruginosa	21	R	R	R	15	26	R	R
S. marcescens	22	R	R	R	16	23	R	R
S. saprophyticus	16	R	R	R	15	15	R	R
P. mirabilis	21	R	R	R	15	10	R	R
K. pneumoniae	15	R	R	R	15	R	15	R
S. aureus	28	R	R	R	R	R	R	R

Key: OFL-Ofloxacin, AUG-Augmentin, CAZ- Ceftazidime, CRX-Cefuroxime, GEN-Gentamicin, CTR-Ceftriaxone, ERY-Erythromycin, CXC-Cloxacillin; R-Resistant, I-Intermediate, S-susceptible.

IV. Conclusion

This study revealed that the fruit extract of *T. tetraptera* commonly used by the local people in Africa in the preparation of herbs, has the potential of being used in the production of drugs with a broad spectrum of activity. This study also serves as an affirmation that the traditional application of sample is of great essence and that it possess antimicrobial properties which can be used for the treatment of a wide range of diseases. The antimicrobial activities of *T. tetraptera* was investigated in this study and proven that it is a potential source of antibiotics for the development of newer and more effective antibacterial agent. With respect to this study, it is recommended that clinical studies should be carried out on this plant to harness its potential for drug production.

Conflict of Interest: We have no conflict of interest.

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Introduction

FMRC/AMRC is the most prestigious membership of Global Journals accredited by Open Association of Research Society, U.S.A (OARS). The credentials of Fellow and Associate designations signify that the researcher has gained the knowledge of the fundamental and high-level concepts, and is a subject matter expert, proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice. The credentials are designated only to the researchers, scientists, and professionals that have been selected by a rigorous process by our Editorial Board and Management Board.

Associates of FMRC/AMRC are scientists and researchers from around the world are working on projects/researches that have huge potentials. Members support Global Journals' mission to advance technology for humanity and the profession.

FMRC

Fellow of Medical Research Council

FELLOW OF MEDICAL RESEARCH COUNCIL is the most prestigious membership of Global Journals. It is an award and membership granted 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. Fellows 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 Fellow Members.

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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A FMRC member gets access to a closed network of Tier 1 researchers and scientists with direct communication channel through our website. Fellows can reach out to other members or researchers directly. They should also be open to reaching out by other.

Career | Credibility | Exclusive | Reputation



Certificate

Certificate, LoR and Laser-Momento

Fellows 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.

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Fellows can use the honored title of membership. The “FMRC” is an honored title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., FMRC or William Walldroff, M.S., FMRC.

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All the Fellow members of FMRC get a badge of "Leading Member of Global Journals" on the Research Community that distinguishes them from others. Additionally, the profile is also partially maintained by our team for better visibility and citation. All fellows get a dedicated page on the website with their biography.

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Certificate

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Preferred Author Guidelines

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

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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 or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

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

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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.
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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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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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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.

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Preparing your Manuscript

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

Manuscript Style Instruction (Optional)

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

Structure and Format of Manuscript

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

A research paper must include:

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

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

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

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.



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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.



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

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

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

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 though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

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

Final points:

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

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

The discussion section:

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

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.



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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.



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Results:

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

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

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

Content:

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

What to stay away from:

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

Approach:

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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.

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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.

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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.

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

Approach:

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

Describe generally acknowledged facts and main beliefs in present tense.

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

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