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EDITORIAL

With this issue, THE SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY completes twenty three (23) years of continuous, uninterrupted publication. The printed version of the JOURNAL has been distributed in more than fifty (50) countries of the globe, covering all continents.

We publicly thank all the members of the Editorial Board that includes distinguished scientists from all continents for the assistance, advice and financial help given during the last twenty three (23) years.

At the present, because of poor health we can not continue as Editor.

Professor Dr. Luis Brandini De Boni is assuming as Editor in Chief of the SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY.

Prof. Dr. Lavinel G. Ionescu.

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
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SCAN ME

CARACTERIZAÇÃO HIDROGEOQUÍMICA DOS ÍONS PREDOMINANTES NAS ÁGUAS SUBTERRÂNEAS DA MINA 3 (COMPANHIA NITROQUÍMICA BRASILEIRA), MORRO DA FUMAÇA, SANTA CATARINA, BRASIL

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ABSTRACT

This paper presents a comparative hydrogeochemical characterization on the predominant ions of the underground water that flow the Cocal vein, in the Mina 3 (Companhia Nitroquímica Brasileira), Fluorine Distritic of Santa Catarina, Morro da Fumaça Co., Santa Catarina, Brazil. Four samples were selected: P_1 – underground water to 300 m of depth; P_2 – underground water to 150 m of depth; P_3 – water of the dam of reject; P_4 – treated water of provisioning of Companhia Catarinense de Águas e Saneamento (CASAN). In the water sample collected in the selected sources, quantitative determinations of physical-chemical parameters (pH and electric conductivity) and of cations (Al, Ca, Cl, Li, Mg, Mn, K, Si, Na, and Zn) and anions (sulfate, chloride, bicarbonate, and fluorine) were performed. The results obtained revealed that most of the analyzed ions are related to the representatives originated from rocks that constitute the pools where the studied water flow. The hydrogeochemical classification was obtained starting from the miliequivalence pattern among the main elements, in the cations and anions form,

being used the triangular diagram of Piper. The underground water of the point 1 presented a hydrogeochemical characterization compatible with is geological environment occurrence and being perceptible the characterization of an interrelation among the rock and the water that circulate within and they also presented an amount of larger ions than the water of the point 2, in since it flow through a mineralized pool. The underground water of the point 2 presented good potable conditions, with some restrictions. In the water of the point 3 there is a larger ions concentration by due to the washing of the mineral, that allows a larger solubilization of the elements, and the time of permanence of the water in the lake also causes larges concentration. The water collected in P_4 point exhibit good water potability parameters and absence of conditions that favor fluorosis.

RESUMO

O artigo apresenta a caracterização hidrogeoquímica dos íons predominantes nas águas de percolação do filão Cocal, Mina 3 (Companhia Nitroquímica Catarinense), Distrito Fluorítico de Santa Catarina, Morro da Fumaça, Santa Catarina, Brasil. Foram selecionados quatro pontos de amostragem: P_1 – águas subterrâneas a 300 m de profundidade; P_2 – águas subterrâneas a 150 m de profundidade; P_3 – águas da barragem de rejeito; P_4 – água de abstecimento da mina, tratada pela Companhia Catarinense de Águas e Saneamento (CASAN). Nas amostras coletadas nos pontos selecionados foram realizadas determinações quantitativas de parâmetros sísico-químicos (pH e condutividade elétrica) e de cátios e ânions predominantes (Al, Ca, Cl, Li, Mg, Mn, K, Si, Na, Zn, sulfato, cloreto, bicarbonato e fluoreto). Os resultados obtidos revelaram que a maioria dos íons analisados apresentam relação direta com os constituintes advindos das rochas que constituem os aquiferos por onde as águas circulam. A classificação hidrogeoquímica foi obtida a partir da relaçao de miliequivalência entre os elementos principais, na forma de cátios e ânions, utilizando-se o diagrama triangular de Piper. As águas P1 apresentaram

caracterização hidrogeoquímica compatível com seu ambiente de ocorrência, sendo perceptível a caracterização de uma interrelação entre as rochas e as águas que nelas circula; nas águas P2 a quantidade de íons foi menor do que em P1, pelo fato de circularem em um aquífero mais mineralizado; as águas P_3 apresentam uma concentração de íons maior, fator que se deve à lavagem do minério, com consequente maior solubilização dos elementos químicos, além do que, uma maior concentração é devido ao maior tempo de permanência das mesmas no lago; as águas P4 apresentam bons parâmetros de potabilidade, além de ausência de condições que favoreçam fluorose.

INTRODUCTION

Os organismos podem privar-se de água de alimentação por poucos dias, entretanto privando-os de água os mesmos podem sucumbir em poucas horas. Nas últimas décadas têm-se notado uma crescente preocupação em relação à escassez dos recursos hídricos. Em áreas industrializadas encontra-se uma forte marca das atividades humanas na qualidade química das águas. Este fato é em particular marcante onde predominam aquíferos passíveis de serem influenciados pelas atividades antrópicas. Nas proximidades dos grandes centros urbanos temos, principalemente, problemas associados às descargas de poluentes. Em áreas agrícolas, a química da água pode estar fortemente influenciada pelos produtos químicos utilizados e nas regiões de mineração temos um caso especial de indústria que acarreta um impacto significativo sobre a qualidade das águas subterrãneas. O homem dispõe naturalmente de dois contingentes de águas disponíveis para uso imediato: as águas de escoamento superficial, disponíveis em rios e lagos (cerca de 3%) e as águas subterrâneas (cerca de 97%)[1,2]. As características das águas subterrâneas vrefletem os meios por onde elas percolam, guardando uma estreita relação com os tipos de rochas drenadas e com os produtos das atividades humanas, adquiridos ao longo de seu trajeto. Todas as águas na natureza possuem, em graus distintos, um conjunto de sais em solução, sendo que as águas subterrâneas possuem, em geral, teores mais elevados do que as águas de superfície, por estarem intimamente expostas aos materiais solúveis presentes nos

solos e nas rochas. A quantidade e o tipo de sais presentes nestas águas dependem, em consequência, do meio percolado, do tipo e velocidade do fluxo subterrâneo, da fonte de recarga do aquífero e do clima vigente na região.

Este trabalho apresenta os resultados obtidos em análises feitas em amostras de água provenientes de quatro pontos de referência da Mina 3 (Companhia Nitroquímica Catarinense), Morro da Fumaça Co., Santa Catartina, Brasil.

As amostras P_1 e P_2 foram coletadaa respectivamente a 300 e 150 m de profundidade, nas galerias da mina, que percolam respectivamente o filão de fluorita Cocal e as coberturas sedimentares sobrejacentes ao Granito Pedras Grandes; P_3 - água do lago de decantação (barragem de rejeitos) da mineradora; P_4 – água de abastecimento da mineradora, tratada pela Companhia Catarinense de Águas e Saneamento (CASAN). Foram analisados dez cátios (alumínio, cálcio, ferro, lítio, magnésio, manganês, potássio, silício, sódio e zinco) e quatro ânios (bicarbonato, cloreto, fluoreto e sulfato), além do pH e da condutividade elétrica dessas águas.

Os objetivos do trabalho foram: caracterizar as águas provenientes da Mina 3 quanto aos aspectos físico-químicos e quanto aos íons predominantes; classificar hidrogeoquimicamente as águas dos quatro pontos considerados; verificar se a concentração dos parâmetros analisados nas águas para consumo humano atendem aqueles estabelecidos pela resolução do CONAMA (n^0 396, 03.04.2008)[3]; comparar a potabilidade da água que abastece o município de Morro da Fumaça com as águas subterrâneas servidas na Vila Mina Fluorita (moradores da mineradora).

LOCAL ESTUDADO

O presente estudo foi desenvolvido no local geologicamente identificado como filão Cocal ($28^0$36'42"S; $49^0$15'58"W), Distrito Fluorítico de Santa Catarina, Bacia do rio Urussanga, Folha de Criciúma (SH.22-X-B-IV-1), Companhia

Nitroquímica Catarinense (Grupo Votorantim), Morro da Fumaça, Santa Catarina, Brasil.

GEOLOGIA

O embasamento do Distrito Fluorítico da Santa Catarina é constituído predominantemente pelo Granito Pedras Grande[4], que localmente é cortado por diques de rochas subvulcânicas ácidas do Eo-Paleozóico[5]. Também ocorrem rochas da Bacia do Paraná (formações Rio do Sul e Rio Bonito – arenitos e lamitos), além de diques e soleiras de diabásio da Formação Serra Geral. Todas estas litologias são cortadas pelo filão Cocal (fluorita), que se encaixa preferencialmente no granito (Figure 1).

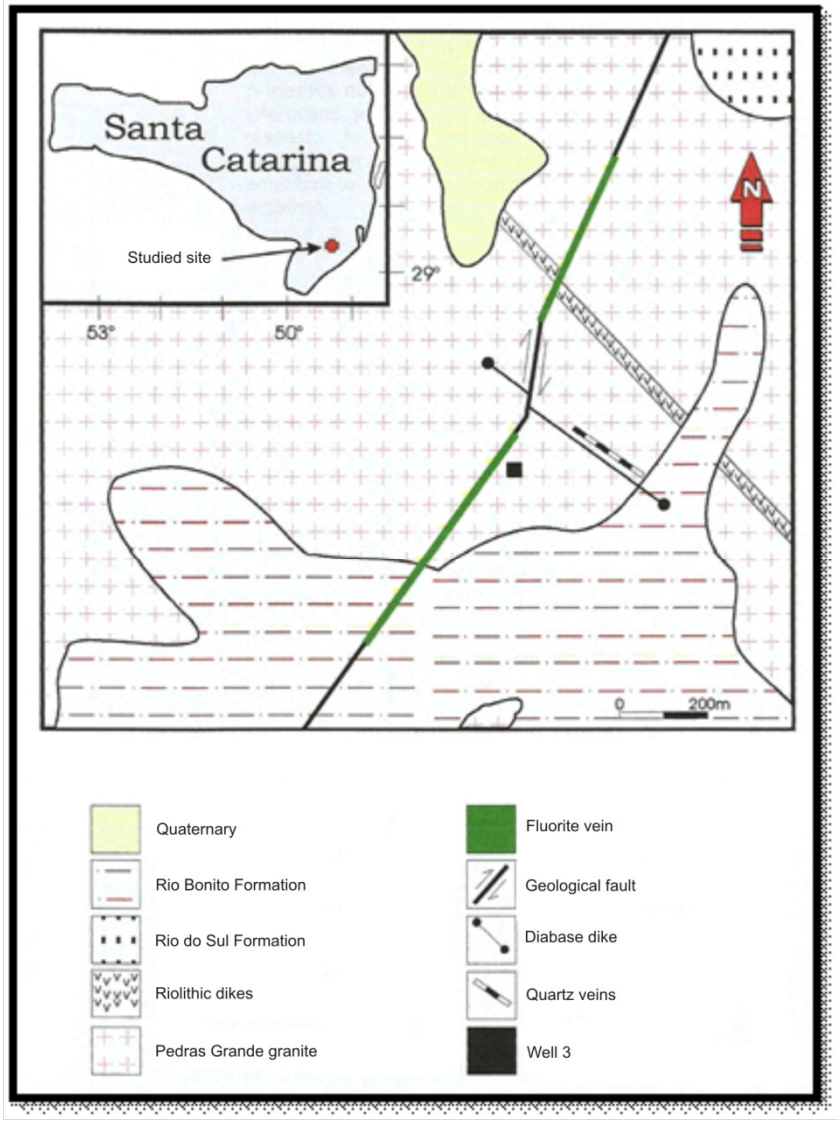

Figure 1 – Geological map of the study location (modified)[6].

Os principais minerais ocorrentes ao longo da Mina 3 são: fluorite (CaF_2), illite ($(K,H_3O)(Al,Mg,Fe)_2(Si,Al)_4O_{10}[(OH)_2,H_2O$)-montmorillonite (smectite) ($(Na,Ca)_{0,3}(Al,Mg)_2Si_4O_{10}(OH)_2.nH_2O$), barite ($BaSO_4$), quartz ($SiO_2$), pyrite ($FeS_2$), goethite (α-FeO(OH)), hematita (Fe_2O_3) e psilomelane (a mixture of manganese oxides)[6-7].

MATERIAIS E MÉTODOS

A etapa de campo constou de uma visita à Mina 3 (Companhia Nitroquímica Brasileira), no município de Morro da Fumaça, para reconhecimento da mineradora e coleta das amostras de água.

As amostras foram coletadas em grascos de polietileno com tampa, com capacidade de 1 L cada. Depois de coletadas, as mesmas foram armazenadas em ambiente de refrigeração até o instante da realização dos procedimentos analíticos, que seguiram a preconização do Standad Methods[8]. As amostras foram subdivididas em volumes menores, sendo que as destinadas às determinações de cátions foram preservadas em ácido nítrico concentrado. Para as determinações dos parâmetros físico-químicos e as análises de cátios e ânions, foram utilizados os seguintes equipamentos: estufas, sistema ultrapurificador de água, forno de mufla, balanças analíticas, pHmetro de bancada, condutivímetro, sensor de temperatura e eletrodo seletivo para fluoreto, chapas de agitação, espectômetro de absorção atômica, forno de grafite e fotômetro de chama. Para eliminar possíveis contaminantes iônicos a vidraria foi previamente lavada e imersa em solução de ácido nítrico 0.1 $mol.L^{-1}$.

As amostras sofreram digestões ácidas (branda (para metais solúveis) e enérgica (para metais totais)) com o intuíto de disponibilizar em matrizes solúveis os elementos metálicos constituintes. Após, segui-se os procedimentos analíticos com as diversas determinações paramétricas que visaram avaliar o comportamento de cada elemento analisado e suas interações com os demais elementos em relação ao meio percolado por essas águas.

RESULTADOS

Os resultados obtidos nos procedimentos analiticos estão apresentados nas tabelas abaixo:

Tabela 1 – Parâmetros físico-químicos

	Point 1	Point 2	Point 3	Point 4
pH	**7.35**	**6.53**	**7.33**	**6.50**
Condutividade elétrica ($\mu S.cm^{-1}$)	**500**	**200**	**516**	**712**

Tabela 2 – Teor de ânions ($mg.L^{-1}$)

	Point 1	Point 2	Point 3	Point 4
Dicarbonate	**99.30**	**52.30**	**131.40**	**19.00**
Clorine	**69.42**	**23.14**	**54.00**	**15.42**
Fluorine	**7.31**	**1.67**	**14.29**	**0.42**
Sulfate	**0.36**	**0.25**	**0.45**	**0.14**

Tabela 3 – Teor de cátions disponíveis ($mg.L^{-1}$)

	Point 1	Point 2	Point 3	Point 4
Aluminum	**0.307±0.012**	**0.207±0.006**	**7.053±0.006**	**0.273±0.006**
Calcium	**30.467±0.231**	**10.882±0.017**	**26.900±0.100**	**2.709±0.002**
Iron	**0.540±0.005**	**0.275±0.004**	**17.160±0.010**	**0.672±0.004**
Lítio	**0.108±0.002**	**0.039±0.00**	**30.108±0.002**	**0.019±0.002**
Magnesium	**1.893±0.006**	**2.303±0.006**	**3.947±0.006**	**1.917±0.006**
Manganese	**0.045±0.001**	**0.027±0.001**	**0.581±0.001**	**0.027±0.001**
Potassium	**2.090±0.000**	**1.184±0.000**	**7.321±0.000**	**1.285±0.000**
Silicon	**18.041±0.165**	**18.356±0.098**	**29.686±0.197**	**5.603±0.204**
Sodium	**46.516±0.000**	**15.206±0.000**	**61.308±0.000**	**9.585±0.000**
Zinc	**0.088±0.00**	**10.314±0.010**	**0.563±0.001**	**10.974±0.006**

Tabela 4 - Teor de cátions disponíveis ($mg.L^{-1}$)

	Point 1	Point 2	Point 3	Point 4
Aluminum	0.537±0.015	0.397±0.006	20.757±0.012	0.390±0.000
Calcium	63.167±0.076	13.032±0.010	36.100±0.000	3.117±0.002
Iron	1.094±0.002	1.163±0.004	26.400±0.017	0.934±0.003
Lítio	0.130±0.002	0.050±0.000	0.228±0.000	0.040±0.003
Magnesium	2.060±0.000	2.787±0.006	5.290±0.000	2.940±0.000
Manganese	0.051±0.001	0.062±0.002	0.769±0.001	0.062±0.002
Potassium	10.703±0.065	1.687±0.000	16.677±0.000	1.486±0.000
Silicon	39.035±0.480	42.540±0.383	51.765±0.345	11.876±0.718
Sodium	63.882±0.000	21.792±0.000	107.568±0.000	13.325±0.000
Zinc	10.688±0.006	15.195±0.006	0.841±0.00	11.363±0.006

Todas as águas subterrâneas contém sais em dissolução em maiores concentrações do que nas águas superficiais, devido a uma maior exposição das mesmas nos extratos geológicos aos materiais solúveis. Os tipos de sais e suas concentrações dependem do meio ambiente, do fluxo a da fonte de água subterrânea. Estes sais originam-se da dissolução dos materiais rochosos pelos quais estas águas percolam.

O conteúdo de sais nas águas subterrâneas deve-se a diversos fatores. A concentração inicial nas águas que escoam no solo para serem infiltradas no aquífero é influenciada por fatores climáticos. Ao infiltrarem as mesmas corroboram para a dissolução dos sais na porção não saturada dos aquíferos (zona de aeração), que serão carreados para a zona saturada dos mesmos. A concentração neste compartimento dependerá dos tipos litológicos dos mesmos e do tempo de escoamento. Evaporação de água próximo a superfície dos mesmos é o fator primordial de enriquecimento em sais das águas subterrâneas[9].

O pH das águas subterrâneas é controlado pelo teor de CO_2 dissolvido nas mesmas e o de carbonatos[1]. O pH geral das águas minerais do estado de Santa

Catarina oscila entre 4.6-8.6 e o pH; já o pH das água minerais provenientes de rochas cristalinas varia entre 4.6-7.9[9]. O pH das águas estudadas (Tabela 1) corresponde aos valores arbitrados para as águas minerais de Santa catarina e depende do aquífero em que elas permeiam, sendo que a solubilização dos componentes presentes pode alterar o valor. Isto explica a diferença entre as águas subterrâneas do Ponto 1 (águas que permeiam os filões mineralizados) e as do ponto 2 (águas que permeiam as rochas sedimentares), já que as mesmas provém de aquíferos com diferentes composições litológicas (Figure 2)

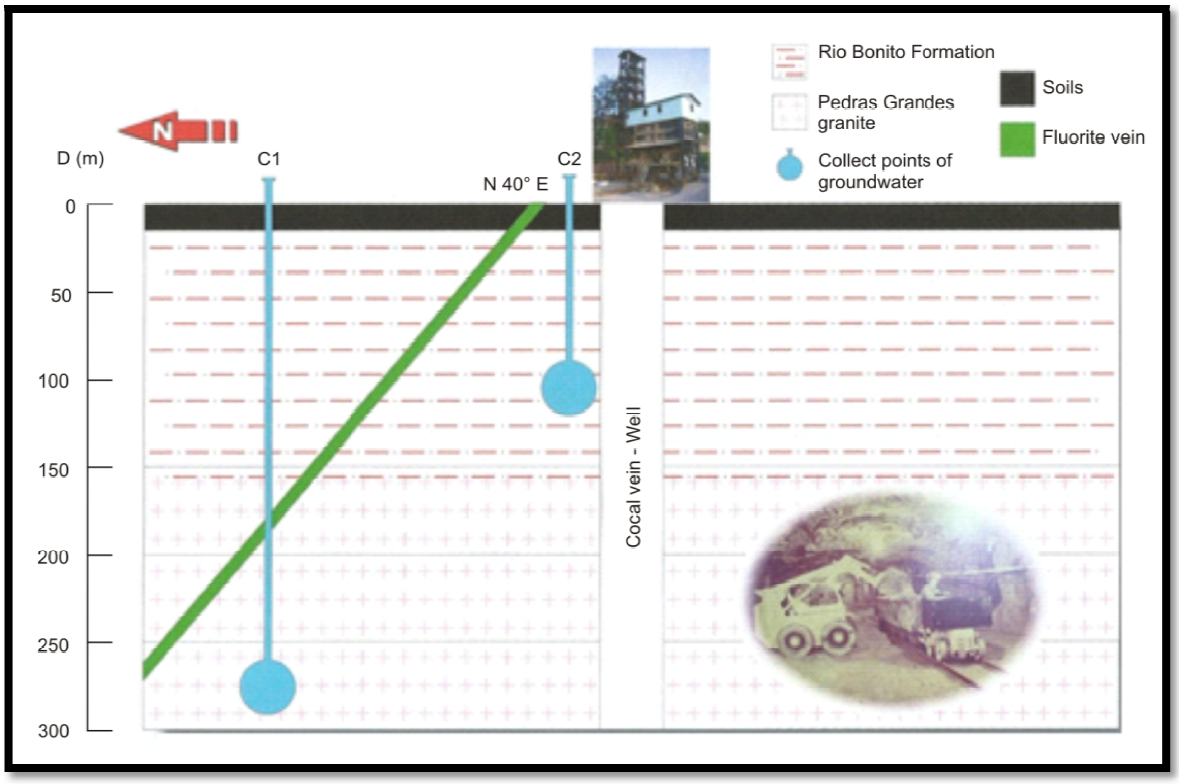

Figure 2 – Perfil esquemático do Cocal vein e pontos de coleta das águas subterrâneas (P_1 e P_2).

A água do ponto 3 (lago de decantação) é proveniente da água de lavagem do minério, que provém do Ponto 1. Assim justifica-se a diferença mínima de pH entre estes dois pontos. Os teores de carbonato e bicarbonato estão diretamente relacionados ao pH. Como o pH das águas analisadas provenientes dos pontos da mina encontram-se entre 6.53 e 7.35, isso significa que as águas não apresentam

carbonatos, pois águas com estes ânions tem pH acima de 8.30. Portanto, as águas da Mina 3 apresentam somente bicarbonatos, que se encontram presentes a partir de pH 4.50. Em condições normais a grande maioria das águas que circulam em aquíferos cristalinos apresentam condições de potabilidade sem restrições, com pH entre 6.5 e 7.5, portanto em condições de neutralidade[10]. Na Mina 3, os poços com entradas de água acima de 150 m, geralmente apresentam pH entre 6.5 e 7.9, podendo eventualmente alcançarem valores superiores a 8, sempre que os teores de Ca forem inferiores a 10 ppm ou carbonato de cálcio inferiores a 100 ppm. Nos poços onde as entradas de água se deram abaixo ou próximo de 200 m de profundidade, o pH sobe, alcançando em média valores na ordem de 9. Logo, em relação ao pH as água analisadas são neutras.

Em soluções diluídas, como as águas subterrâneas, a condutibilidade elétrica é proporcional à quantidade de minerais dissolvidos nas mesmas, aumentando com o aumento de temperatura[1]. A condutividade geral das águas minerais do estado de Santa Catarina varie entre 6.15 e 1.15 $\mu S.cm^{-1}$, o que reflete o grau de mineralização das águas. Os valores de condutividade elétrica nas águas subterrâneas da Mina 3 (Tabela 1) indicam haver intensa atividade iônioca no Ponto 1 em relação ao Ponto 2, o que se justifica pelo fato das destas (Ponto 1) estarem em contato direto com o filão de florita, ou seja um aquífero mais rico em fases minerais. Águas altamente mineralizadas podem receber incidência de radioatividade, o que se dá, principalmente, pela presença de radônio[9]. Águas que percolam rochas cristalinas tendem a ser mais ricas em minerais radioativos. Assim o alta condutividade elétrica dectada para as águas que circulam pelo Granito Pedras Grandes justifica os altos teores verificados para a condutividade elétrica (Figura 3).

Análises químicas em 80% das águas que circulam aquíferos cristalinos apresentam teor de bicarbonato entre 40 – 80 ppm[10]. A presença tanto de carbonato quanto de bicarbonato se reflete diretamente na alcalinidade, que por definição é a caspacidade de uma água em neutralizar ácidos. A alcalinidade das águas minerais de Santa Catarina variam de 154.57 $mg.L^{-1}$ (129.3 – 189.3) para as

rochas da Formação Serra Geral e 121.46 mg.L^{-1} (33.5 – 209.8) para as rochas do embasamento cristalino[9]. Nas águas subterrâneas da Mina 3 o teor encontrado para bicarbonato é similar ao encontrado para as rochas cristalinas do estado de Santa Catarina, que podem conter também bicarbonato proveniente das rochas da Formação Serra Geral, que estão presentes também no distrito Fluorítico de Santa Catarina. O bicarbonato é o ânion presente em maior concentração nos pontos 1, 2 e 3. Isto está associado às altas concentrações de Ca e Na, o que pode ser decorrente da solubilização de bicarbonatos. A maior concentração no Ponto 3, significa o incremento do teor durante o processo de lavagem do minério que se acumula no lago de decantação da mina.

O teor de cloreto de uma água subterrânea expressa a quantidade de íons Cl presentes, que podem advir da dissolução de $CaCl_2$, NaCl, KCl e $KMgCl_3.6H_2O$. Uma água é considerada satisfatória quando o teor de cloreto é menor do que 150 ppm. Portanto, os teores das águas da Mina 3 encontram-se dentro desta faixa.

O íon fluoreto, geralmente, é encontrado nas águas subterrâneas em pequenas concentrações. O mesmo deriva da fluorite (CaF_2), principal halogeneto presente em rochas ígneas, ou então de minerais nos quais o flúor possa estar presente como fluorapatite ($(CaPO_4)_3F$), topaz ($Al_2SiO_4F_2$), mica and tourmaline. O flúor liberado pelo intemperismo destes minerais ocorre na forma de íon fluoreto, que apresenta alta mobilidade. O mesmo pode formar complexos estáveis com Al, Fe, B e Ca. Assim, no ciclo geoquímico o flúor pode ser removido das águas pela coprecipitação com óxidos secundários de Fe, podendo também ser complexado com Fe e Al na forma de fosfatos. Em águas que percolam litologias cristalinas a presença de flúor é mais significativa em face dos granitoides apresentarem teores mais altos do elemento. Nas águas minerais do estado de Santa Catarina, que percolam o Granito Pedras Grandes, os teores variam de 0,40 – 1,10 mg.L^{-1}[9]. Nas águas subterrâneas da Mina 3 (pontos 1 e 2) (Tabela 2) o teor de fluoreto está acima daqueles das águas minerais do estado de Santa Catarina. Certamente isto se deve à presnça de fluorita, pois seus filões cortam as litologias do Distrito Fluorítico de Santa Catarina (Figuras 2 – 3), encaixando-se preferencialmente no Granito Pedras Grandes. A concentração deste ânion no Ponto 1 está acima dos teores indicados por Hausman[10], porém os teores do ponto 2 estão de acordo com

a faixa do 5 ppm[3]. O teor desejável de fluoreto nas águas deve estar em torno de 1,00mg.L^{-}[1-3], pois acima deste valor favorece à fluorose, patologia dentária que afeta principalmente crianças, promovendo manchas escuras no esmalte dentário e crescimento protuberante nos dentes incisivos[11]. As águas de consumo humano (Ponto 4) apontam 0.42 mg.L^{-1}, estando assim dentro dos parãmetros de segurança em relação à fluorose.

Sulfatos em águas subterrâneas são provenientes, principalmente, da presença de gipsita ($CaSO_4.2H_2O$) e anidrita ($CaSO_4$), fases minerais que já foram identificadas nas litologias da Formação Serra Geral[1-12]. Os teores de sulfato das águas coletadas no Ponto 1, também podem provir da solubilização da pirita, fase mineral encontrada amplamente na Mina 3, associada à fluorita[6-9]. O mesmo pode ser aludido à presença do ânion no Ponto 2.

O alumínio encontra-se presente em rochas ígneas, geralmente, na forma de Al_2O_3, com teor entre 10-20%[14],. Seus íons hidratados são de extrema importância na regulação do pH de águas subterrâneas. Em meios moderadamente ácidos o alumínio disponível, na forma iônica é convertido em hidróxido de alumínio com estruturas que podem variar de $Al(OH)^{2+}$ a $[Al_{10}(OH)_{22}]^{8+}$, processo que ocorre concomitante com outros cátions como o ferro e o manganês. Deste modo pode ocorrer alterações do pH do meio. A intensidade do processo está diretamente relacionada com as quantidades do elemento presentes nas formas disponíveis, além da intensidade da interação entre o corpo aquoso e o leito pelo qual flui. As concentrações de alumínio em águas subterrâneas podem variar entre 100mg.L^{1-} a 2000mg.L^{1-}[13]. O alto teor de alumínio nas águas de rejeito (P_3) (tabelas 3-4) pode ser atribuído, em parte, as interações entre a água subterrânea e os sedimentos. O aumento da concentração de alumínio no ponto 3 em relação a águas do ponto 2, contraria a teoria do aumento da sua concentração em função do valor do pH, já que estas águas apresentam praticamente os mesmos pH. Isto pode significar que algum outro fator, ou elemento químico, esteja sendo mais determinante. As concentrações do elemento presentes nas águas da Mina Fluoral em termos de concentração total

(Tabela 4), podem indicar que parte do alumínio presente neste meio não esteja disponível. O teor de alumínio presente no ponto 2 encontra-se dentro da faixa indicada por Allen (1974) [(13)]. Já, as águas do ponto 1, encontram-se acima desta faixa. Isto pode estar relacionado à composição da rocha que forma o aqüífero, podendo a mesma conter minerais com o elemento em sua constituição, ou, ainda, estes minerais serem muito pouco solúveis.

O cálcio é o quinto elemento químico mais abundante na crosta terrestre, com 3.63%[(14)]. As principais fontes do elemento são os plagioclásios cálcicos, calcite, dolomite, flurite, apatites and anfiboles. Na forma de carbonato de cálcio é pouco solúvel em égua. Ocorre, portanto, nas águas na forma de bicarbonato e sua solubilidade se dá na quantidade de gás carbônico dissolvido. Nas águas subterrâneas as variações de temperatura e pressão modificam o CO_2 dissolvido, o que reflete sobre o conteúdo em cálcio. Estas variações, ora levam à solubilização do carbonato de cálcio, ora levam a sua precipitação. Segundo Allen (1974)[(13)], em águas naturais o cálcio encontra-se na faixa de 1 a 100mg.L^{1-}. O teor em cálcio nas águas do ponto 1 (Tabelas 3 e 4) pode estar relacionado com a presença de fluorite, que é o principal mineral constituinte do filão por onde estas águas percolam. A diferença entre a concentração de cálcio total e disponível indica que grande parte dele não está solubilizado, podendo estar precipitado ou associado a outros elementos. A atividade e disponibilização do cálcio são favorecidos numa faixa de pH entre 6,5-8,5 (Brady, 1979) [(13)]. Assim, podee ser justificada a diferença na concentração de cálcio entre os pontos 1 e 2, que também pode estar associada à composição mineral do aqüífero correspondente às águas do ponto 2, que percolam rochas sedimentares.

O ferro, quarto elemento em abundância na crosta terrestre, com 5.00%[(14)], está presente em todas as águas potabilizáveis. O ferro comumente se encontra presente nas águas na forma de Fe^{2+} e Fe^{3+}. O íon ferroso é instável em presença do ar, mudando para o estado férrico quando a água que o contém é exposta, sendo que pode dissolver-se em quantidade de cerca de 50ppm, em águas neutras e em maiores proporções se as águas forem levemente ácidas. Já o íon férrico tende à insolubilidade em águas alcalinas ou fracamente ácidas. O alto teor de ferro nas águas do ponto 3 pode estar relacionado ao contato da mesma com as tubulações

da Mina, já que a mesma é utilizada para a lavagem do minério. A diferença entre a quantidade disponível e a total, pode significar que parte do ferro está na forma insolúvel, o que pode ter sido causado, também, pela ação de microorganismos presentes no lago de decantação ou pelo contato com o ar, podendo estar na forma de óxidos insolúveis. Segundo Hausman[10], os teores mais elevados de Fe ocorrem quando as águas dos granitóides são contaminadas pelas águas que circulam pelas formações sedimentares sobrepostas. Isto pode justificar a diferença entre os teores de ferro do ponto 1 e do ponto 2 (Tabelas 3 e 4). O ferro nas águas do ponto 1 pode ter a sua fonte principal nos minerais goethite (α-$Fe^{3+}O(OH)$, hematite (α-Fe_2O_3) e pyrite (FeS_2), presentes na composição rochosa do aquífero[6]. Concentrações entre 1-5 mg.L^{1-} de ferro em ´paguas subterrâneas são comuns[1]. Após a aeração, o teor pode cair a 0.1 mg.L^{1-}. O teor disponível deste elemento nos pontos 1 e 2(Tabela 3) encontra-se abaixo da faixa indicada, podendo ter sido reduzido pela aeração provocada quando do bombeamento da água do aqüífero até a superfície.

O lítio na crosta terrestre é um elemento traço (20 ppm/Kg) [14], o que justifica sua baixa concentração nas águas subterrâneas. Nas amostras dos pontos 1, 2 e 3, os teores de lítio demonstram comportamento diretamente proporcional às variações de pH. A pequena disparidade entre os valores para as concentrações do elemento nas formas solúvel e total, nos pontos 1 e 2 (Tabelas 3 e 4), supõe que praticamente todo o lítio está na forma solúvel. No ponto 3 a concentração praticamente dobrou da forma solúvel para a total, o que significa que boa parte do mesmo não está disponível para o meio, podendo em conseqüência ter ficado retida nos sedimentos.

O magnésio tem uma participação de 2.09% na crosta terrestre[14]. Seu comportamento geoquímico é semelhante ao do cálcio, com a diferença de que forma sais mais solúveis. Encontra-se presente em minerais como a biotite, anfiboles, olivines e pyroxenes, mais estáveis ao intemperismo químico do que aqueles fornecedores de cálcio, por isso a presença de magnésio é menor do que a de cálcio nas águas subterrâneas. A atividade química e a disponibilidade do

magnésio está diretamente ligada ao pH do meio, decrescendo em função da diminuição do mesmo. Assim, em meios mais básicos ou neutros sua disponibilidade tende a ser mais alta. Ávila et al. [9] atribuíram valor de 0.96 mgL^{1-} para aqüíferos da Serra Geral Formation (basaltos), 2.28 mgL^{1-} para aqüíferos do Pedras Grandes Granite 2 e 28.33 mgL^{1-} para as águas da Rio do Sul Formation. Os valores encontrados para o ponto 1, estão de acordo com os valores acima, podendo parte do magnésio encontrado ser proveniente da Rio do Sul Formation, presente junto ao Pedras Grande Granite no Fluoritic District of Santa Catarina. O maior teor de Mg no ponto 2 (Figura 3) pode ser função de sua maior proximidade com a superfície, onde há maior dissolução. A diferença entre a quantidade disponível e a total (Tabelas 3 – 4) pode ser atribuída à facilidade deste elemento formar sais mais solúveis, levando à ilação de que praticamente todo o magnésio esteja solubilizado.

O manganês ocorre na crosta terrestre com teor de 0.09% [14]. Embora, quimicamente, seu comportamento assemelha-se ao do ferro em ocorrência nas águas naturais, seus teores são sensivelmente menores devido a sua ocorrência crustal ser menor do que a daquele elemento. Nos pontos 1, 2 e 3 (Tabelas 2 e 4) do local estudado, o Mn é o elemento de menor concentração. Segundo Hausman [10], em águas que percolam o cristalino os teores em Mn são da ordem de 0.05 ppm, ficando assim os valores obtidos nos pontos 1 e 2 deste estudo inseridos na faixa citada.

O silício é o segundo elemento mais abundante da crosta terrestre (27.72%)[14] . Combinado com o oxigênio, forma o mineral mais difundido na natureza (quartz). Segundo a CETESB[1], a água pode dissolver somente diminutas quantidades de sílica; não obstante as águas subterrâneas podem conter até 100 ppm do óxido., sendo que valores entre 0,5-8 $mg.L^{1-}$ são encontrados para o silício nas águas subterrâneas[13]. Segundo Mason[14], em pH menor que 4 a sílica dissolve lentamente, entretanto entre 5-9 sua solubilidade tende a aumentar consideravelmente. Ávila et al. [9] afirmam que o fator determinante para a variação do teor em sílica nas águas subterrâneas depende da litologia, clima e pH. Nas rochas cristalinas de Santa Catarina (Pedras Grandes Granite) está relacionada com a decomposição química dos minerais silicatados (feldspates,

plagioclases e quartz). Nas águas dos pontos 1, 2 e 3 o Si é o segundo cátion com maior concentração nas águas. O fato de que grande parte dele não se encontra solubilizado deve-se a sua grande resistência a sua desintegração geoquímica (elemento altamente estável). A alta concentração registrada nos meios estudados (Tabela 3), pode ter sido facilitada pelo pH das águas, que se encontram dentro da faixa de solubilidade da sílica. Os teores aqui encontrados estão em consonância com os de Ávila et al. [9] para os do Pedras Grandes Granite.

O sódio e o potássio ocorrem com distribuição geoquímica muito próximas na crosta terrestre, com 2.83 e 2.59 %, respectivamente[14], muito embora sua participação nas águas subterrâneas se d~e em quantidades sensivelmente diferentes. O potássio ocorre na estrutura de muito minerais (micas, feldspates, sylvite), entretanto pode ficar retido nas argilas o que o torna menos disponível para o meio. Sua faixa de concentração em águas subterrâneas se dá na ordem de 0.5 a 10 mg.L^{1-}, logo as águas dos pontos 1 e 2 estão de acordo com esta faixa. Nas água de litologias cristalinas o K ocorre com melhor representatividade, pois as mesmas são muito ricas em feldspates[9]. Segundo Todd[15], as águas subterrâneas que percolam rochas ígneas dissolvem pequenas quantidades de substâncias minerais, devido a relativa insolubilidade da composição destas rochas. Isto pode justificar o alto teor de K na forma total das águas do ponto 1 em relação à forma solúvel deste mesmo ponto, e também, à pequena diferença entre o teor disponível e o teor total na amostragem do ponto 2, já que o aqüífero por onde circulam estas águas é constituído por rochas sedimentares que são mais solúveis do que as ígneas. O sódio é um elemento químico quase que sempre presente nas águas subterrâneas e, deriva de uma série de minerais (feldspates, anfiboles e halite). Por ser muito solúvel em ambientes minerotróficos há uma grande tendência à perda do mesmo por solubilização. Sua atividade não sofre mudanças em função de alterações de pH. Sais de Na formados em processos intempéricos são geralmente muito solúveis. Os valores médios para o Na em águas naturais varia entre 2 – 100 mg.L^{1-} [13]. Nos pontos 1, 2 e 3 das águas da Mina Fluoral observou-se que o na é o cátion com a maior concentração. Isto se justifica pela alta solubilidade do

elemento. O alto teor do íon em concentração total (pontos 2 e 3) – (Tabela 4), significa que grande parte do mesmo não está solubilizado. Segundo Ávila et al.[9], as águas da Serra Geral Formation são as que apresentam os maiores teores do elemento, devido ao fato destas rochas serem muito ricas em feldspates e pyroxenes, minerais ricos em Na.

O zinco participa da composição da crosta terrestre como elemento-traço (70 ppm) [14]. Os principais minerais portadores do elemento são sphalerite (ZnS), zincite (ZnO) e hemimorphite ($Zn_4Si_2O_7(OH)_2.H_2O$), não registrados para a Mina Fluoral. O baixo teor de Zn nas águas do lago de decantação (ponto 3) ,tanto na forma solúvel, quanto na total (Tabelas 3 - 4), pode ser atribuído ao fato de que praticamente todo o zinco presente nas águas do ponto 1 está na forma residual insolúvel, muitas vezes como colóide, tendo se depositado no fundo do lago. Também. Parte do mesmo pode ter sido complexado pelos microorganismos, plantas e matéria orgânica presentes neste ambiente. A alta concentração de Zn na forma total para as águas dos pontos 1 e 2, pode ser explicada por carreamento advindo das tubulações da Mina, pois os minerais de Zn não ocorrem nas litologias locais.

CLASSIFICAÇÃO HIDROGEOQUÍMICA DAS ÁGUAS SUBTERRÂNEAS DA MINA FLUORAL

A classificação das águas subterrâneas foi efetivada através do diagrama trilinear de Piper[10] para as concentrações de Ca^{2+}, Mg^{2+}, K^{+}, HCO_3^{4-}, CO_3^{2-}, Cl^{-} e SO_4^{2-} em $Meq.L^{1-}$ e para os valores de condutividade elétrica nas quatro amostras analisadas. As águas do ponto 1 foram classificadas como cloretadas sódicas; as do ponto 2 como bicarbonatadas calco-sódicas; as do ponto 3 como bicarbonatadas sódicas.

CONCLUSÕES

No presente estudo foram estudados apenas os elementos de maior importância e presentes em quantidades mais significativas na constituição

mineral dos aqüíferos da Mina Fluoral (Mina 3), Morro da Fumaça, Santa Catarina, Brazil.

- O pH das águas ficou dentro de uma faixa considerada neutra;

- os elementos químicos presentes nas águas subterrâneas, em geral, apresentaram atividades diretamente relacionadas às composições minerais dos aqüíferos por onde estas águas circulam;

- o aumento de profundidade de circulação das águas subterrâneas fez aumentar os teores de mineralizações das mesmas, pois à medida que aumenta a profundidade, aumenta o volume de rochas mineralizadas;

- a classificação hidrogeoquímica da águas estudadas demostrou haverem cloretadas sódicas, águas bicarbonatadas sódicas e águas bicarbonatadas calco-sódicas na Mina Fluoral;

- a maior concentração dos elementos químicos analisados deu-se no ponto 3, o que se justifica por serem águas de lavagem do minério, ocorrendo um incremento pela dissolução do mesmo e, também, porque no lago não há movimentação da água, o que3 facilita a sua concentração, além da interação com os sedimentos e os microorganismos existentes no mesmo;

- a maior concentração de cátions na forma total, nas águas do ponto 1 em relação às do ponto 2, é explicada pelo fato de que as águas subterrâneas que passam por rochas ígneas dissolvem pequenas quantidades de substâncias minerais, ao contrário das rochas sedimentares, que são mais solúveis;

- pela comparação das águas do ponto 2 e do ponto 4, juntamente com padrões estabelecidos pelo CONAMA, conclui-se que as ponto 2 apresentam menores condições de potabilidade;

- os valores encontrados nas águas do ponto 1 estão de acordo com os obtidos para as águas minerais do Estado de Santa Catarina, ambas circundantes do Pedras Grandes Granite.

Ao serem analisadas as características hidrogeoquímicas das águas que percola o embasamento cristalino de uma região, deve-se sempre levar em consideração as anomalias relacionadas à contribuição de certos elementos, pois

fatores de ordem tectônica podem induzir, devido a mineralizações, variações na qualidade das águas, elevando em conseqüência os teores de alguns elementos químicos.

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Volatile Components of *Oncoba spinosa* leaf and *Morus mesozygia* leaf and stem bark

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ABSTRACT

The essential oils of *Oncoba spinosa* leaf and *Morus mesozygia* leaf and stem bark were extracted using hydro distillation and analyzed by means of Gas chromatography (GC) and GC coupled with mass spectrometry (GC-MS). The yields of the essential oils were; 0.50 %, 0.165 % and 0.456 % respectively for *Oncoba spinosa* leaf, *Morus mesozygia* leaf and stem oil. A total of twenty eight, thirty four and twenty compounds representing 92.0%, 92.0% and 96.9% of the total oil contents were identified, respectively from the leaf of *O. spinosa*, leaf and stem oil of *M. mesozygia*. Leaf oil of *O. spinosa* contained linalool (22.1 %), β – caryophyllene (18.7 %), caryophyllene oxide (10.6 %) and pentadecanal (5.6 %) as the main constituents. *M. mesozygia* leaf oil was dominated with β – elemene (11.7 %), (*E*) –β- ionone (12.4 %), α- selinene (5.1 %), germacrene A (6.0 %), δ – cadinene (4.7 %) and spathulenol (7.4 %) while *M. mesozygia* stem oil had 2 –dodecanone (77%) and hexahydrofarnesylacetone (13 %) as its main constituents.

Key words: *Oncoba spinosa, Morus mesozygia,* linalool, (*E*) –β- ionone, 2 –dodecanone.

INTRODUCTION

Plants essential oils and their components have been known to exhibit biological activities. Screening of plants extracts or their essential oil for these properties has become of great importance because of their growing interest in food and pharmaceutical industries (1). *Oncoba spinosa*, commonly known as the snuff-box tree is a plant species in the genus *Oncoba* and family *Flucourtiaceae*. It is a small to medium-sized deciduous tree of about 5 m high and has simple leaves. The tree is widely distributed along the eastern side of Africa as far as South Africa, mainly in dry woodland or open savanna in a wide range of sites from river valleys to rocky hills. In African medicine, the roots are used in the treatment of dysentery and bladder complaints and fruits in the treatment of leprosy. The anti-ulcerogenic potential (2), antihyperglycemic and antineoplastic activities (3), and anti-convulsant property (4) have been studied. The fruit was found to be effective in the treatment of urinary tract diseases of cows and camels (5). In addition, the antipasmodial (6) and antihelmintic properties (7) have been discussed in literature.

Morus mesozygia (Moraceae), commonly called black mulberry or African mulberry, is a small to medium sized forest tree. M*orus* comprises of ten to fifteen species with only one specie (*Morus mesozygia*) native to tropical Africa. It has a wide distribution in tropical Africa, from Senegal eastward to Ethiopia and southward to Zambia, Angola, Mozambique and South Africa (8). In African traditional medicine, parts of *M. mesozygia* are used in decoctions, baths, massages and enemas against rheumatism, lumbago, intercostal pain, neuralgia, colic, stiffness,

debility, diarrhea and dysentery. It is evident from literature that extract of the plant is known to have antidepressant property (9). Arylbenzofurans and flavonoids were isolated from *M. mesozygia* (10) and the anti-plasmodial and cytotoxic properties of arylbenzofuran have been studied (11). The leaf essential oil of another species *Morus rotunbiloba* was characterized and dominated by benzyl alcohol (10.48%), dihydroactinolide (10.11%) and palmitic acid (7.01%); the oil also displayed good antibacterial and cytotoxic activities (12).

Therein, we describe the chemical composition of the essential oil from the leaves of *O. spinosa* and leaves and stem bark of *M. mesozygia* for the first time.

EXPERIMENTAL

Plant materials

Plant samples were collected from the Botanical garden, University of Ibadan, Ibadan, Nigeria in March 2014 by Mr. Owolabi. The plants were authenticated at the Herbarium of the Forestry Research Institute of Nigeria (FRIN) Oyo State, Nigeria where voucher specimens FHI 110037 and FHI 110038 for *Oncoba spinosa* and *Morus mesozygia* respectively were deposited. Plant samples were air-dried prior to extraction.

Isolation of essential oils

250 g of each of the dried plant samples were shredded and their oils were obtained by separate distillation for 3 hours in a Clevenger type apparatus. The essential oil fractions obtained were kept in a refrigerator at 4^{o}C prior to analysis.

Analysis of the Oils

Gas chromatography (GC) analysis was performed on a HP-5890 Gas chromatograph equipped with a Flame ionization detector and fitted with HP-5 capillary column (30 m x 0.25 mm, 0.25 µm film thickness). The GC oven temperature was programmed at 60^{o}C (held for 10 min), heated to 220^{o}C at 5^{o}C/min. The injector and detector temperatures were maintained at 250^{o}C. Helium was used as carrier gas at a flow rate of 2 mL/min with a split ratio of 1:30.

Gas chromatography / Mass spectrometry (GC-MS) analysis were carried out on a Varian CP-3800 gas chromatograph interfaced to a Varian Saturn 2000 ion trap Mass Detector operated at 70 eV. The injector and transfer line temperatures were 220^{o}C and 240^{o}C, respectively. The GC oven temperature was programmed from 60 to 240^{o}C at 3^{o}C/min. Helium was used as a carrier gas at a flow rate of 1 mL/min, injection of 0.2 µL aliquots as 10% Hexane solution, split ratio of 1:30.

Identification of the constituents on both columns was based on comparison of the retention times with those of authentic samples, comparing their retention indices relative to the series of n-hydrocarbons, and by comparison of their mass spectra with published spectra and those of reference compounds from NIST 98 and ADAMS (13, 14, 15, 16, 17). The relative concentration of each constituent was calculated by integration of GC peak areas.

RESULTS AND DISCUSSION

A total of 28 compounds amounting to 92% were identified in *O. spinosa* leaf essential oil. Among these are, 29.2% oxygenated monoterpenes, 22.3% sesquiterpenes and 9.2% oxygenated sesquiterpenes. It also contained non-terpene derivatives (14.7%) and apocarotenes

(16.6%). However, the major constituents identified are; linalool (22.1%), β- caryophyllene (18.7%), caryophyllene oxide (10.6%), pentadecanal (5.6%), methyl salicylate (4.2%) and *(E)* - β –ionone (3.8%). Linalool and β- caryophyllene which dominated the oil are noted for many commercial and medicinal applications. Linalool, a naturally occurring terpene alcohol has been utilized in perfumery (18) while β- caryophyllene is a dietary cannabinoid (19) which has been found to have clearly demonstrated its ability in the regulation of emotional beahviour, thereby suggesting that its receptor could be relevant therapeutic target for the treatment of anxiety and depressive disorders (20).

A total of 34 constituents were identified in the leaf of *M. mesozygia*, representing 92.0% of the total fraction. Monoterpenes (0.5%), sesquiterpenes (48.5%), oxygenated sesquiterpenes (22.1%), apocarotenes (18.4%) and non-terpene derivatives were identified in the essential oil. The main constituents are; (*E*)-β-ionone (12.4%), β-elemene (11.7%), Spathulenol (7.4%), α-selinene (5.1%), δ–cadinene (4.7%), humulene epoxide II (3.8%) *(E)* - α-ionone (3.4%), trans-α-bergamotene (3.5%). (*E*)-β-ionone is a flavouring agent and exhibits anti-proliferative function (21) while β-elemene has exhibited tremendous anti-tumor properties (22, 23).

The essential oil of the stem bark of *M. mesozygia* afforded identification of 20 constituents, corresponding to 96.9% of the oil constituents. Monoterpenes (0.1%), oxygenated monoterpenes (0.5%), sesquiterpene (2.5%), oxygenated sesquiterpenes (1.4%), apocarotenes (14.0%) and non-terpene derivative (78.4%) were identified in the essential oil. The main constituents in the oil are; 2-dodecanone (77.0%), hexahydrofarnesyl acetone (13.0%), ar-curcumene (1.0%). Moreover, it was observed that both *M. mesozygia* leaf and stem bark essential oils had *E*- β- ionone (12.4% and 0.4%) respectively as common constituents and also the most abundant in the leaf oil of *M. mesozygia.* Other common constituents are spathulenol

(7.4% and 0.3%), caryophyllene oxide (2.9% and 0.5%) alloaromadendrene (1.5% and 0.3%), *E*-geranylacetone (2.1% and 0.6%), α-humulene (1.1% and 0.2%), β- caryophyllene (1.2% and 0.6%), limonene (0.5% and 0.1%) and 2- pentylfuran (0.5% and 0.3%) respectively. The dominance of the stem oil of *M. mesozygia* by 2-dodecanone and hexahydrofarnesyl acetone has indicated that the bark oil may exhibit excellent insect repellant properties. In a study by Ndungu et al (24), it was revealed that 2-dodecanone was one of the constituents in the oil of *Cleome monophylla* that exhibited high repellent activity against tick and maize weevil. In another related work, it was deduced that 2-dodecanone exhibited good protection efficacy against *An. gambiae* (100% protection at 10% concentration) and also concluded that methyl ketone analogies are potential source of insect repellent including mosquitoes (25). Hexahydrofarnesylacetone is also a widespread ketone found in plant and insects which several insect uses as part of their pheromone bouquet (26).

The abundance of both compounds 2-dodecanone and hexahydrofarnesylacetone totaling 90% of the stem oil of *M. mesozygia* gives a strong lead that it possesses good insect repellent properties and has therefore opened an avenue to research into the repellent ability of the essential oil to different species of insects. On the contrast, these compounds were not found in the leaf oil yet confirming a known fact that plant tissues differ in their chemical constituents. The constituents of these oils were at variance to the compounds characterized in the oil of another species *Morus rotunbiloba*, fifty-one compounds were detected in the leaf essential oil with benzyl alcohol (10.48%), dihydroactinolide (10.11%) and palmitic acid (7.01%) being the most abundant (12). Out of the compounds characterized in *M. rotunbiloba*, only four were found in

either of the two oils of *M. mesozygia*, these are E-α-ionone, hexahydrofarnesylacetone, geranylacetone and β-cyclocitral.

CONCLUSION

The different therapeutics and commercial importance of the characterized compounds in these essential oils justifies the use of different parts for the treatment of different ailments. This represents the first attempt to our knowledge at characterizing the essential oil constituents of the leaves of *O. spinosa* and leaves and stem bark of *M. mesozygia*.

Table 1: Essential Oil Constituents of the Leaves of *Oncoba spinosa* and Leaves and stem bark of *Morus mesozygia*.

S/N	Constituents	L.R.I[a]	L.R.I[b]	*O. spinosa* Leaves	*M. mesozygia* Leaves	*M. mesozygia* Stem bark
1	*(E)* -2-hexenal	865	846	0.8	-	-
2	*p*-xylene	867	883[a]	-	0.5	-
3	6-methyl-5-heptene-2-one	987	992	-	-	0.4
4	2-pentyl furan	993	984	0.7	0.5	0.3
5	*(E)* -2-pentenyl furan	1004	1000[b]	0.7	-	-
6	Limonene	1032	1024	-	0.5	0.1
7	1,8-cineole	1034	1026	-	-	0.3
8	Linalool	1101	1095	**22.1**	**-**	-
9	Nonanal	1103	1100	-	0.5	-

10	α –terpeneol	1191	1186	3.1	-	-
11	Methyl salicylate	1192	1190	4.2	0.5	-
12	β-cyclocitral	1222	1217	0.9	0.5	-
13	Nerol	1230	1227	1.2	-	-
14	Methyl carvacrol	1246	1298	-	-	0.2
15	Geraniol	1256	1249	2.8	-	-
16	α-copaene	1377	1374	0.7	2.9	-
17	β-elemene	1392	1389	0.9	**11.7**	-
18	2-dodecanone	1395	1394	-	-	**77.0`**
19	*cis*- α-bergamotene	1416	1411	-	0.5	-
20	β-caryophyllene	1419	1417	**18.7**	1.2	0.6
21	α- santalene	1420	1416	-	1.3	-
22	*(E)*- α-ionone	1428	1428	1.1	3.4	-
23	Trans-α-bergamotene	1438	1432	-	3.5	-
24	Precocene1	1448	1440[d]	-	-	0.3
25	epi-β-santalene	1450	1445	-	0.5	-
26	α-humulene	1455	1452	1.1	1.1	0.2
27	*(E)*-geranylacetone	1455	1453	1.5	2.1	0.6
28	Alloaromadendrene	1462	1461	-	1.5	0.3
29	Cabreuva oxide B	1463	1462	0.8	-	-
30	γ-muurolene	1478	1478	-	1.8	-

31	Germacrene D	1482	1484	-	1.3	-
32	ar-curcumene	1483	1479	-	-	1.0
33	*(E)*-β-ionone	1487	1487	3.8	**12.4**	0.4
34	10,11-epoxycalamenene	1492	1476[e]	-	-	0.4
35	α-selinene	1496	1498	-	5.1	-
36	Germacrene A	1504	1508	-	6.0	-
37	β-bisabolene	1509	1505	-	2.0	0.3
38	β-curcumene	1513	1514	0.9	2.3	
39	δ-cadinene	1524	1513	**-**	**4.7**	-
40	(*E*)- γ –bisabolene	1534	1529	-	0.5	-
41	Dihydroactinolide	1536	1531[f]	1.9	-	-
42	α- calacorene	1543	1544	-	0.6	-
43	*(E)*-nerolidol	1565	1561	1.8	-	-
44	Caryophyllene alcohol	1569	1568	-	-	0.6
45	*(Z)*-3-hexenyl benzoate	1570	1564[g]	0.5	-	-
46	Spathulenol	1577	1577	0.7	**7.4**	0.3
47	Caryophyllene oxide	1582	1582	**10.6**	2.9	0.5
48	Viridiflorol	1591	1592	1.1	-	-
49	Humlene epoxide II	1607	1608	0.8	3.8	-
50	Tetradecanal	1612	1611	1.2	-	-

51	Humulene-1,6-diene-3-ol	1618	1619[h]	-	1.9	-
52	Cedrenol	1630	I642[i]	-	1.7	
53	Caryophylla-4(14),8(15)-dien-5-ol	1637	1623[j]	1.1	-	-
54	Selin-11-en-4- α-ol	1655	1658	-	1.9	-
55	α-bisabolol	1684	1685	-	0.7	-
56	Pentadecanal	1716	1711[k]	**5.6**	-	-
57	Hexahydrofarnesyl acetone	1845	1843[l]	**-**	**-**	**13.0**
58	Benzyl salicylate	1864	1864	-	-	0.4
1	Monoterpene hydrocarbons			0.0	0.5	0.1
2	Oxygenated monoterpenes			29.2	0.0	0.5
3	Sesquiterpenes hydrocarbons			22.3	48.5	2.5
4	Oxygenated sesquiterpenes			9.2	22.1	1.4
5	Apocarotenes			16.6	18.4	14.0
6	Non-terpene derivatives			14.7	2.5	78.4
	Total Identified			**92.0**	**92.0**	**96.9**

"Notes"

L.R.I[a] = Linear Retention Index From This Work

L.R.I[b] = Linear Retention Index From Literature (Adams)

^[d-l] = References 27-35.

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THE CHEMICAL AND MEDICINAL POTENTIALS OF THE FRUIT ESSENTIAL OIL OF *Chrysophyllum cainito* (INDIA STAR APPLE)

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ABSTRACT

Chrysophyllum cainito is a lesser known fruit with several medicinal applications. The essential oil was obtained by hydrodistillation and was analysed by gas chromatographys/mass spectrometry, GC/GC-MS. Twenty components were identified in the essential oil, the oil was characterized by a high proportion of fatty acids (69.37%) represented by pyruvic acid, 10-hendecenoic acid, E-9-tetradecenoic acid, pentadecanoic acid, hexadecacanoic acid and cis-9-octadecenoic acid. The oil yield was 0.19 v/w of the wet sample and its compositional profile showed markedly qualitative and quantitative variation with essential oil from Cuba. This essential oil was seen to be active against gram-positive bacteria, Escherichia coli, Klebsiella pneumoniae, Samonella typhimurium and Proteus mirabilis and also gram negative bacteria, Staphylococcus aureus and Streptococuss agalactiae.

KEYWORDS: ***Chrysophyllum cainito, essential oil, hydrodistillation, fatty acid***

RESUMO

O óleo essencial do fruto de Chrysophyllum cainito foi obtido por hidrodestilação e analisado com cromatografia gasosa e espectrometria de massa (GC-MS). Um total de vinte constituintes foram identificados no óleo essencial.que foi caracterizado pela presença alta de ácidos graxos (69.37%). Os componentes principais foram os ácidos piruvico, 10-hexadecenoico, E-9-tetradecenóico, pentadecenóico, hexadecanóico e cis-9-octadecenóico. O rendimento foi 0.19 volume/peso da amostra molhada e o perfil composicional foi muito diferente do óleo essencial de Cuba tanto qualitativamente quanto quantitativamente. O óleo essencial mostrou atividade antimicróbica tanto contra bactéria gram- positiva com Escherichia coli, Klebsiella pneumoniae, Salmonella typhimurium e Proteus mirabilis qauanto gram- negativa como Staphylococcus aureus e Streptococcus agalactiae.

PALAVRAS CHAVE:Chrysophyllum cainito, Óleo essencial, Hidrodestilação, Ácidos graxos

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INTRODUCTION

Traditional and folklore medicine plays an important role in health care services around the globe. Plants are known to synthesize a wide range of chemical substances, many of which have been and can be of tremendous value in treatment and prevention of diseases. Man has depended on the plants and plants extract as a source of medicine, food, shelter, clothing etc. since creation [15].

Herbal drugs have been one of the oldest practiced by mankind because of their cheap availability and wide application, scientist in various parts of the country concentrate on studies of these remedies in order to use these as an alternative to expensive imported drugs [12] several pharmaceutical companies are engaged in the development of natural product drugs through the isolation of the so-called active molecule from plant extracts. It is estimated that today, plant material are present in, or have provided the models for 50% western drugs [14].

Chrysophyllum cainito commonly called star apple belongs to the family sapotaceae, it usually comes in two forms, either the dark purple skinned variety with red-purple pulp or the green skinned variety with clear white pulp. In Nigeria, this fruit is not commonly eaten like the African star apple fruit, *chrysophyllum albidum.* This plant is a medicinal plant which contain substances that could be used for therapeutic purposes of which are precursors for the synthesis of useful drugs [1].

In Ivory Coast, decoction of the leaves is used for the treatment of hypertension, while in Cuba, a decoction of the leaves is used as a cancer remedy, while that of the bark is used as an antitussive (cough suppressant) [9]. In Eastern part of Africa, infusion of the leaves have been used against diabetes and articular rheumatism, while the bark is considered a tonic and stimulant. In Venezuela, the fruit and the seed are used as a diurectic and the ripe fruits because of their mucilaginous character are eaten to sooth inflammation, laryngitis and pneumonia. The latex of the tree in Brazil is applied on abscesses [7]. In Nigeria, few people eat the fruit because it is not common but those that eat it do so because of the sweetness and its mucilaginous character.

Chrysophyllum cainito belongs to the family *sopotaceae* and their chemical and biological studies are scarce [2] and there is lack of chemical information about the genus *chrysophyllum,* but a number of reports concerning the isolation and characterization of bioactive compounds from various parts of the plant have appeared in the literature.

The volatile constituents of star apple were extracted in Cuba [10], the essential oil were analyzed by GC-MS and one hundred and four compounds were identified in the aroma concentrate of which *(E) -2-hexanal, limonene, linalool, copaene and hexadecanoic acid* were found to be the major constituents.

In another study from Florida, fresh fruit of chrysophyllum cainito were extracted with methanol and partitioned with hexane and ethyl acetate sequentially. Nine known polyphenolic antioxidants were identified and epicatechin is present in highest concentration [6].

The aqueous extract of the leaves was found at a dose of 20g/l to reduce the hyperglycemia of diabetic rat from 5g/l to 1.4g/l [5].

The extract of *chrysophyllum cainito* was used in goats against *Haemonchos contostus*[4] it was reported that the extract has nematocidal activity and this confirmed its use in ethnoveterinary practices and animal health management [11].

The purpose of the present investigation therefore was to extract, analyse and determine the antimicrobial activity of the essential oil of this lesser known fruit of *chrysophyllum cainito.*

MATERIALS AND METHODS

Plant material and essential oil isolation

Fresh fruits of *chrysophyllum cainito* (dark purple skinned variety) were collected from the campus of Ajayi Crowther University, Oyo in South West Nigeria in March, 2013. The sample was authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, where voucher specimens were deposited and was given voucher number FHI 109475. A sample of 700g was pulverized using mortar and pestle. The fresh fruit was subjected to hydro-distillation method using an all glass Clevenger apparatus according to procedure described by European Pharmacopoeia [18]. The oil was collected and kept in refrigerator without further treatment until GC/GC-MS analysis.

Gas Chromatography/GC mass spectrometric analysis

The chemical composition of the essential oil was analysed using GC-MS technique. The mass spectrometer was SHIMADZU GCMS-QP2010 plus (Shimadzu Corporation, Japan) in the electron impact (EI) ionization mode (70ev) and HP 5MS (bonded 0.25μm capillary column (restek, Bellefonte, PA) Injector and detector temperature was held at 60°C for 30 minutes then programmed to 240°C at rate of 5°C/min. Helium (99.99%) was the carrier gas at a flow rate of 1ml/min. Diluted samples (1/100 in hexane v/v) of 1.0ml were injected automatically, the linear velocity of the column was 36.8cm/sec, each peak was then analysed and assigned a number in the order that it was detected. The identification of the components was based on comparison of their mass spectra with those of NIST library, mass spectra database and literature.

Antimicrobial Activity Tests

The antibacterial activities of the essential oil was measured against gram-negative (*Escherichia coli, Klebsiella pneumoniae, Salmonella typhimurium and Proteus mirabelis* and gram-positive *Staphylococcus aureus and Streptococcus agalactiae* using a well diffusion method according to the National Committee for Clinical Laboratory Standard [8]. Briefly, Petri plates containing approximately 25-30ml of nutrient agar medium were swabbed using cotton applicator with a 24 hours sub-cultured bacterial strains which were prepared in dilution to match the turbidity intensity of the MacFarland Standard. Wells (6mm diameter) were punched in the agar and filled with 10μl of the extracts of different concentrations (1000, 100 and 10μgml^{-1}). The plates were incubated at 37°C for 24 hours. The antibacterial activities were assessed by measuring the inhibition zone diameter (mm) around the well.

RESULTS AND DISCUSSION

Hydro distillation of the fresh fruit of *chrysophyllum cainito* produced a clear light essential oil. The oil yield is 0.19% v/w of the wet sample. The chemical components identified by GC/GC-MS are listed in Table 1. It was characterized by a high proportion of fatty acids (69.37%) represented by (IIz) –II hexadecenoic acid (24.42%), cis-9-octadecenoic acid (oleic acid) (13.04%), E-9- Tetradecenoic (12.21%), E-9-Hexadecanoic acid (9.54%), Pentadecanoic acid (3.18%), Nonanoic acid (pelargic acid) (3.18%), Pyruvic acid (2.97%) and 10-Hendecenoic acid (0.83%).

The compositional profile of the fruit oil showed markedly qualitative and quantitative variation with that from Cuba. *(E)-2-hexanal, limonene, linalool and copaene* which are part of the major constituents of the oil from Cuba were not identified from the Nigerian oil sample, but hexadecanoic acid which was reported as one of the major constituents [10] is present in the Nigerian sample (9.54%).

The oil is seen at different concentration to be active against gram-positive bacteria (K*lebsiella pneumoniae, Salmonella typhimurium and Proteus mirabilis* and also gram-negative bacteria *Staphylococcus aureus and Streptococcus agalactiae* as listed in Tables 2 and 3 and this can be compared with standard antibiotics as listed in Tables 4 and 5.

Table 1. Chemical Constituents of the fruit essential oil of *chrysophyllum cainito*

S/N	COMPONENT	RI	% COMPOSITION
1	3-ethoxy-1-butane	620	0.80
2	Divinyl sulfide	650	2.97
3	2,2-Dimethylpentanal	821	0.80
4	2-pentanethiol	837	2.97
5	Heptan-3-one (ethylbutylketone)	853	0.80
6	Tert-Butylcarbinol	876	0.80
7	Pyruvic acid	919	2.97
8	Monochloromethylisopentanoate	946	0.80
9	Allyl pentanoate	974	2.97
10	Methylcyclooctane	1020	0.83
11	2(3H)-Furanone	1061	2.97
12	(4Z)-4-Methyl-4-undecene	1199	0.83
13	Nonanoic acid (pelargic acid)	1272	3.18
14	10-Hendecenoic acid (Sevinon)	1461	0.0
15	E-9-Tetradecenoic acid	1777	12.21
16	Pentadecanoic acid (pentadecyclic acid)	1869	3.18
17	Hexadecanoic acid (palmitic acid)	1968	9.54
18	(11z)-11-Hexadecenoic acid	1976	24.42
19	E-2-Octadecadecen-1-0l	2061	12.21
20	Cis-9-octadecenoic acid (oleic acid)	2175	13.04
21	Methyl(13E)-13-docosenoate	2483	0.83
	Total		99.95%

Table 2.

Zones of inhibition (mm) showing the antimicrobial activities of the essential oil at different concentrations (Gram-negative bacteria)

Organism	*Escherichia coli*	*Klebsiella pneumoniae*	*Salmonella typhimorium*	*Proteus mirabilis*
Conc (μgml^{-1})	1000 100 10	1000 100 10	1000 100 10	1000 100 10
Zone of inhibition	15 08 09	13 13 13	12 12 12	13 13 13

Keynote –= no inhibition, 6-9mm = low inhibition 10-15mm = moderate inhibition≥15mm = high inhibition.

Table 3.

Zones of inhibition (mm) showing the antimicrobial activities of the essential oil at different concentrations(Gram-positive bacteria).

Organism	Staphyococcus aureus	Streptococcus agalatiae
Conc (μgml^{-1})	1000 100 10	1000 100 10
Zone of inhibition	17 13 13	15 15 15

Keynote –= no inhibition, 6-9mm = low inhibition 10-15mm = moderate inhibition≥15mm = high inhibition.

Table 4.

Zones of inhibition (mm) showing the antimicrobial activity of antibiotics (Gram –negative bacteria)

Organism / Antibiotics/ concentrations	*Escherichia coli*	*Klebsiella pneumoniae*	*Salmonella typhimorium*	*Proteus mirabilis*
Nitrofurantoin (200μg)	25	20	15	15
Ofloxacin (5μg)	20	20	22	33
Gentamicin (10μg)	24	15	18	16

Keynote –= no inhibition, 6-9mm = low inhibition 10-15mm = moderate inhibition≥15mm = high inhibition.

Table 5.

Zones of inhibition (mm) showing the antimicrobial activities of antibiotics (Gram –positive bacteria)

Organism / Antibiotics/ concentrations	*Staphylococcus Aureus*	*Streptococous agalactiae*
Nitrofurantoin (200μg)	28	11
Ofloxacin (5μg)	20	-
Gentamicin (10μg)	-	-

Keynote –= no inhibition, 6-9mm = low inhibition 10-15mm = moderate inhibition≥15mm = high inhibition.

CONCLUSION

The results obtained in this study showed that the fruit essential oil of *chrysophyllum cainito* from South West Nigeria has a qualitative and quantitative variation from the one obtained in Cuba and this can be traced to the tropical nature of Nigeria. This study is the first in Nigeria, to the best of my knowledge.

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SCAN ME

SYNTHESIS AND BIOLOGICAL ACTIVITIES EVALUATION OF SOME NEW SPIRO 1,2,4-TRIAZOLE DERIVATIVES HAVING SULFONAMIDE MOIETY

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ABSTRACT

A series of new spiro 1,2,4-triazoles **V-IXa-j** were synthesized by the reaction of appropriate amidrazones **IV** with cyclic ketones in the presence of *p*-toluene sulfonic acid as a catalyst. The structures of the synthesized compounds have been confirmed by the elemental analysis and spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR and MS). The microbial features of the synthesized compounds were studied using well-established methods from literature.

KEYWORDS: Amidrazone, nitrilimines, spiro 1,2,4-triazole, sulfonamide, cyclic ketone.

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GRAPHICAL ABSTRACT

Synthesis and Biological Activities Evaluation of Some New Spiro 1,2,4-Triazole Derivatives Containing Sulfonamide Moiety

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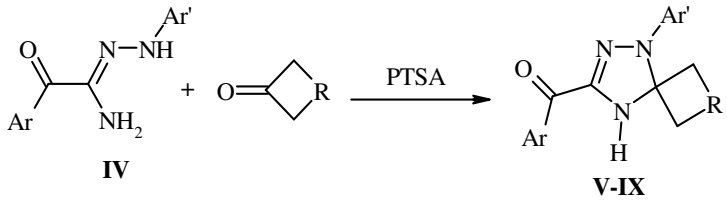

IV **V-IX**

V, Ar = Me; **VI**, Ar = Ph; **VII**, Ar = 2-Furyl;
VIII, Ar = 2-Tienyl; **IX**, Ar = 2-Naphthyl

1. Introduction

Amidrazones represent a class of substances with interesting biological properties. It has been established that they can exhibit antibacterial and antifungal [1-3], antitumor [4], or antituberculosis activities [5]. They were also found as effective herbicides [6], pesticides [7], and insecticides [8]. The interest in amidrazones and their derivatives stems not only from their biological relevance but also from their applications as precursors and intermediates for the synthesis of many heterocyclic compounds [9-11] or as ligands in coordination chemistry. Amidrazones synthesized from different hydrazonoyl halides are reported to react with α-haloesters in the presence of triethylamine as a base under reflux afforded 1,3,5-substituted 4,5-dihydro-1,2,4-triazin-6-ones [12]. Several studies, involving the formation and investigation of biological activities of some spiroheterocyclic compounds having triazole, tetrazine thiadiazole, thiadiazines, thiazolidinone and triazine moieties [13-17]. Recently, unknown dispiroheterocycles containing triazole and tetrazine moieties have been reported by Dalloul [14-17].

Sulfonamide derivatives exhibit a range of bioactivities, including anti-angiogenic, anti-tumor, anti-inflammatory and anti-analgesic, anti-tubercular, anti-glaucoma, anti-HIV, cytotoxic, anti-microbial and anti-malarial agents [18]. Taking into account all previous commentaries of the biological activities of sulfonamides and in continuation of our study on the synthesis of biologically active heterocycles [19-21], efforts have been made to synthesize a series of new spiro heterocycles containing 1,2,4-triazole and piperidone derivatives via cyclization of amidrazones having sulfonamide moiety with cyclic ketones in anticipation of expected interesting biological activities.

2. Results and Discussion

The precursors of amidrazones hydrazonoyl halides **I** employed in this study were prepared according to reported literature procedures [22-25]. Treatment of the hydrazonoyl halides **I** with sodium azide in presence of tetrabutylammonium iodide at room temperature gave azidohydrazones **II** (Figure). The obtained hydrazonoyl azides **II** reacted with triphenylphosphine afforded phosphonimines **III** (Figure). It was found that the hydrolysis of compounds **III** with aqueous hydrochloric acid gave the triphenylphosphine oxide and the amidrazones **IV,** respectively (Figure). The most plausible mechanism for this acid hydrolysis is the one that involves initial protonation of nitrogen followed by the attack of oxygen on the phosphorous atom [22].

The condensation of amidrazones **IV** with cyclic ketones in refluxing dioxane in the presence of catalytic amount of *p*-toluenesulfonic acid produced spiro 4,5-dihydro-1H-1,2,4-triazole derivatives containing sulfonamide moiety **V-IXa-j** (Figure) in good yields. The reaction progress was monitored by TLC to find out the completion of the synthesis.

2.2. Spectral data analysis of compounds IV and V-IXa-j.

The structure of the newly synthesized amidrazones **IV** was elucidated on the basis of their spectroscopic data and elemental analyses. The electron impact (EI) mass spectra of these compounds **4a-j** displayed the correct molecular ions ($M^{+\cdot}$) in accordance with the suggested structures. The IR spectra exhibit typical stretching absorption bands of C=O of conjugate ketone, anilide and ester groups at about 1725-1650 cm^{-1}, NH's in the region of 3470-3220 cm^{-1} and 1150, 1060 cm^{-1} attributed to SO_2 of sulfonamide group. The ^1H NMR spectra of these amidrazones DMSO-d_6 show three characteristic signals, as singlet at 12.6-12.4 ppm (SO_2NH), singlet of the NNH group in the region of 6.3-6.0 ppm and singlet of NH_2 near 4.9-5.0 ppm. In addition the characteristic singlet signal of the CONH group in compounds containing anilide group was observed at about 10.8-9.8 ppm and the expected proton signals of the aromatic rings in the range of 8.4-6.9 ppm. The ^{13}C NMR spectra exhibit characteristic signals for the Ar-C=O carbon at about 192-159 ppm and for the C=N moiety at about 143-141 ppm.

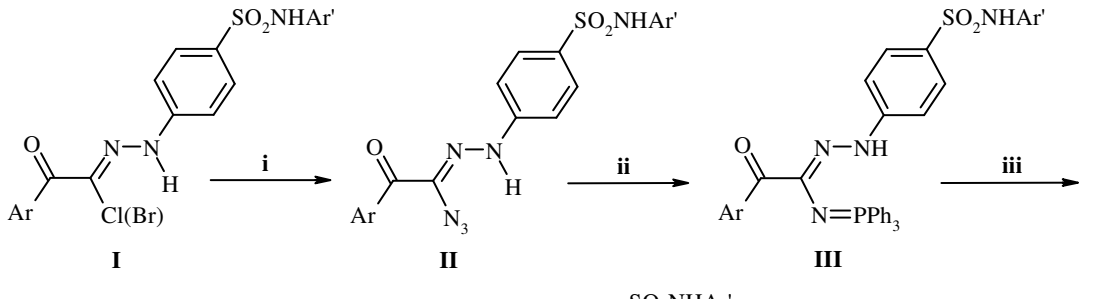

Figure. Synthetic pathway for the preparation of compounds **IV** and **V-IXa-j.**

The structural assignment of the prepared spiro 1,2,4-triazoles **V-IXa-j** was based on elemental analysis and spectral data. The electron impact (EI) mass spectra of these compounds **4a-j** displayed the correct molecular ions ($M^{+\cdot}$) in accordance with the suggested structures. Physical properties, molecular ion peaks and microanalysis are presented in experimental section. The IR spectra of these compounds revealed the presence of NH band of dihydrotriazole ring resonated near 3370-3350 cm^{-1}. In addition to the bands of characteristic functional groups. The ^{1}H NMR spectra of products **V-IXa-j** in DMSO-d_6 display a characteristic singlet in the region of 5.6-5.5 ppm due to NH proton of dihydrotriazole ring. Also, the spectra exhibit a characteristic singlet at 12.7-12.6 ppm due to SO_2NH proton. The ^{13}C NMR spectra display characteristic signals of the suggested structures. The signal at 90-95 ppm, which is attributed to C-5 (spiro carbon) of dihydrotriazole ring is of special significance. This is similar to reported values of spiro carbons flanked by two nitrogens in five-membered heterocycles [23,26]. The spectral data of the obtained compounds **IVa-j** are summarized in the experimental section.

2.2. Antimicrobial activity

The standard nutrient agar disc diffusion method[35,36] was followed to determine the activity of the synthesized compounds against the sensitive organisms *Euterococci, Escherichia coli*, *Staphylococcus aureus*, *Klebsiella spp*, *Proteus spp*, as bacterial strains and two species of fungi, namely *Aspergillus niger*, *Candida albicans*. The compounds tested at a concentration of 1 mg mL^{-1} in N,N-dimethyl formamide (DMF) solution, and measuring the average diameter of the inhibition zone in mm. The results showed that all the tested compounds exhibited a marked degree of activity against bacteria and fungi compared with well-known antibacterial and antifungal substances such as tetracycline and fluconazole. According to NCCLS [27], zones of inhibition for tetracycline and fluconazole < 14 mm were considered resistant, between 15 and 18 mm were considered weakly sensitive and > 19 mm were considered sensitive. Also, the results showed the degree of inhibition varied with the tested compounds (Table). The thiazolyl and pyrimidinyl moieties generally led to dramatic improvements in activity against both bacteria and fungi. The present study can lead medicinal chemists to design and synthesize similar compounds with enhanced biological potency in future.

Table. Antimicrobial screening results of the tested compounds

Cpd. No.	Diameter of the inhibition zone in mm*						
	Antibacterial activity					Antifungal activity	
	Euterococci	*Escherichia coli*	*Staphylo aureus*	*Klebsiella spp*	*Proteus spp*	*Candida albicans*	*Aspergillus niger*
Va	16	17	17	13	16	15	14
Vb	13	18	15	11	10	17	18
Vc	19	15	11	14	16	18	16
Vd	18	16	17	18	19	16	12
VIb	16	19	16	19	11	19	11
VIIa	13	12	14	16	17	16	19
VIIb	19	15	11	14	16	18	16
VIIIa	18	16	17	18	19	16	12
VIIIb	16	19	16	19	11	19	11
IXa	13	12	14	16	17	16	19
DMF	–	–	–	–	–	–	–

*Calculated as average of three values.

3. EXPERIMENTAL SECTION

3.1. Reagents and Instrumentation

Melting points were determined on an A. Krüss Melting Point Meter equipped with a thermometer and are uncorrected. The IR spectra were measured as potassium bromide pellets using a Satellite 3000 Mid infrared spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 300 MHz spectrometer at room temperature in DMSO-d_6 solution using tetramethylsilane (TMS) as internal reference. Chemical shifts were recorded as δ values in parts per millions (ppm) downfield from internal TMS. Electron impact (EI) mass spectra were run on a Shimadzu GCMS-QP1000 EX spectrometer at 70 eV. All compounds were analyzed satisfactorily for C, H and N. The amidrazones **IV** were prepared according to literature procedures [22-25]. Sodium azide, triphenylphosphine and tetrabutylammonium iodide were obtained from Fluka Chemie Company, Switzerland. Sulfathiazole, sulfadiazine, dioxane, tetrahydro-furan (THF) and triethylamine were purchased from Avocado Research Chemicals, England, and used without further purification.

3.2. Synthesis of spiro 4,5-dihyro-1,2,4-triazole derivatives V-IXa-j.

To a stirred solution of the appropriate amidrazone **IV** (10 mmol) and the respective cycloalkanone (20 mmol) in dioxane (50 mL), the catalytic amount of *p*-toluenesulfonic acid (0.1 g) was added. The reaction mixture was refluxed to the completion (monitoring the reaction progress by TLC). The excess of the solvent was evaporated

and the residue was triturated with methanol or ethanol. The resulting crude solid product was collected and recrystallized from ethanol. The following compounds were prepared using this method:

3-Acetyl-1-[4-(thiazol-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.4]non-2-ene (Va); Yield: 65%; m.p.: 197-199 ^{o}C; IR (KBr) ν_{max}: cm^{-1} 3382, 3355 (NH's), 1695 (CH_3-C=O), 1622 (C=N), 1346, 1175 (SO_2); ^{1}H NMR ($CDCl_3$): δ 11.70 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 4H, Ar-H), 8.71 (d, 1H, thiazole), 6.62 (d, 1H, thiazole), 5.65 (s, 1H, NH triazole ring), 2.45 (*s*, 3H, $COCH_3$), 2.37-1.60 (m, 8H, cyclopentane); ^{13}C NMR ($CDCl_3$): *δ* 192.3 (C=O), 169.1-116.5 (Ar-C, C=N and thiazole-C), 92.8 (spiro-C), 34.9, 24.9 (cyclopentane-C), 26.5 (CH_3); MS: *m/z* 405 [M^+]; Anal. Calcd. for $C_{17}H_{19}N_5O_3S_2$ (405.50): C, 50.35; H, 4.72; N, 17.27; Found: C, 50.57; H, 4.60; N, 17.15.

3-Acetyl-1-[4-(pyrimidin-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.4]non-2-ene (Vb): Yield: 71%; m.p.: 216-218 ^{o}C; IR (KBr) ν_{max}: cm^{-1} 3374, 3350 (NH's), 1693 (CH_3-C=O), 1626 (C=N), 1346, 1175 (SO_2); ^{1}H NMR ($CDCl_3$): δ 11.74 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 4H, Ar-H), 8.41 (d, 2H, pyrimidine), 6.83 (t, 1H, pyrimidine), 5.63 (s, 1H, NH triazole ring), 2.55 (*s*, 3H, $COCH_3$), 2.37-1.60 (m, 8H, cyclopentane); ^{13}C NMR ($CDCl_3$): *δ* 192.1 (C=O), 168.19-110.6 (Ar-C, C=N and pyrimidine-C), 92.6 (spiro-C), 34.8, 24.6 (cyclopentane-C), 26.6 (CH_3); MS: *m/z* 400 [M^+]; Anal. Calcd. for $C_{18}H_{20}N_6O_3S$ (400.46): C, 53.99; H, 5.03; N, 20.99; Found: C, 54.23; H, 4.92; N, 21.10.

3-Acetyl-1-[4-(thiazol-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.5]dec-2-ene (Vc): Yield: 66%; m.p.: 204-206 ^{o}C; IR (KBr) ν_{max}: cm^{-1} 3369, 3357 (NH's), 1695 (CH_3-C=O), 1624 (C=N), 1346, 1175 (SO_2); ^{1}H NMR ($CDCl_3$): δ 11.76 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 4H, Ar-H), 8.71 (d, 1H, thiazole), 6.65 (d, 1H, thiazole), 5.65 (s, 1H, NH triazole ring), 2.54 (*s*, 3H, $COCH_3$), 2.07-1.16 (m, 10H, cyclohexane); ^{13}C NMR ($CDCl_3$): *δ* 192.4 (C=O), 168.4-116.2 (Ar-C, C=N and thiazole-C), 92.1 (spiro-C), 35.9, 24.8, 23.2 (cyclohexane-C), 26.5 (CH_3); MS: *m/z* 419 [M^+]; Anal. Calcd. for $C_{18}H_{21}N_5O_3S_2$ (419.53): C, 61.53; H, 5.05; N, 16.69; Found: C, 61.70; H, 4.96; N, 16.55.

3-Acetyl-1-[4-(pyrimidin-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.5]dec-2-ene (Vd): Yield: 65%; m.p.: 192-194 ^{o}C; IR (KBr) ν_{max}: cm^{-1} 3377, 3357 (NH's), 1693 (CH_3-C=O), 1628 (C=N), 1346, 1175 (SO_2); ^{1}H NMR ($CDCl_3$): δ 11.71 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 4H, Ar-H), 8.40 (d, 2H, pyrimidine), 6.82 (t, 1H, pyrimidine), 5.61 (s, 1H, NH triazole ring), 2.55 (*s*, 3H, $COCH_3$), 1.98-1.16 (m, 10H, cyclohexane); ^{13}C NMR ($CDCl_3$): *δ* 191.9 (C=O), 168.25-110.4 (Ar-C, C=N and pyrimidine-C), 91.8 (spiro-C), 35.8, 24.6, 23.1 (cyclohexane-C), 26.4 (CH_3); MS: *m/z* 414 [M^+]; Anal. Calcd. for $C_{19}H_{22}N_6O_2S$ (414.49): C, 55.06; H, 5.35; N, 20.28; Found: C, 54.78; H, 5.50; N, 20.39.

3-Acetyl-1-[4-(thiazol-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.6]undec-2-ene (Ve): Yield: 67%; m.p.: 178-180 ^{o}C; IR (KBr) ν_{max}: cm^{-1} 3375, 3358 (NH's), 1695 (CH_3-C=O), 1625 (C=N), 1346, 1175 (SO_2); ^{1}H NMR ($CDCl_3$): δ 11.76, (s, 1H, NH), 8.27-7.06 (*m*, 4H, Ar-H), 8.70 (d, 1H, thiazole), 6.64 (d, 1H, thiazole), 5.63 (s, 1H, NH triazole ring), 2.54 (*s*, 3H, $COCH_3$), 2.35-1.42 (m, 12H, cycloheptane); ^{13}C NMR ($CDCl_3$): *δ* 192.4 (C=O), 168.2-116.5 (Ar-C, C=N and thiazole-C), 91.6 (spiro-C), 39.4, 28.1, 22.3 (cycloheptane-C), 26.5 (CH_3); MS: *m/z* 433[M^+]; Anal. Calcd. for

$C_{19}H_{23}N_5O_3S_2$ (433.55): C, 52.64; H, 5.35; N, 16.15; Found: C, 52.45; H, 5.24; N, 16.03.

3-Acetyl-1-[4-(pyrimidin-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.6]undec-2-ene (Vf): Yield: 64%; m.p.: 188-190 °C; IR (KBr) ν_{max}: cm^{-1} 3373, 3365 (NH's), 1695 (CH_3-C=O), 1620 (C=N), 1346, 1175 (SO_2); ^1H NMR ($CDCl_3$): δ 11.69 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 4H, Ar-H), 8.36 (d, 2H, pyrimidine), 6.81 (t, 1H, pyrimidine), 5.62 (s, 1H, NH triazole ring), 2.56 (*s*, 3H, $COCH_3$), 2.34-1.40 (m, 12H, cycloheptane); ^{13}C NMR ($CDCl_3$): *δ* 191.8 (C=O), 168.25-110.4 (Ar-C, C=N and pyrimidine-C), 39.1, 28.3, 22.5 (cycloheptane-C), 91.8 (spiro-C), 26.4 (CH_3); MS: *m/z* 428 [M^+]; Anal. Calcd. for $C_{20}H_{24}N_6O_3S$ (428.52): C, 56.06; H, 5.65; N, 19.61; Found: C, 55.86; H, 5.53; N, 19.47.

3-Acetyl-1-[4-(thiazol-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.7]dodec-2-ene (Vg): Yield: 70%; m.p.: 166-168 °C; IR (KBr) ν_{max}: cm^{-1} 3373, 3361 (NH), 1696 (CH_3-C=O), 1627 (C=N), 1346, 1175 (SO_2); ^1H NMR ($CDCl_3$): δ 11.71 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 4H, Ar-H), 8.72 (d, 1H, thiazole), 6.61 (d, 1H, thiazole), 5.65 (s, 1H, NH triazole ring), 2.55 (*s*, 3H, $COCH_3$), 2.42-1.24 (m, 14H, cyclooctane); ^{13}C NMR ($CDCl_3$): *δ* 192.3 (C=O), 168.05-116.5 (Ar-C, C=N and thiazole-C), 90.9 (spiro-C), 41.9, 36.5, 27.2, 21.8 (cyclooctane-C), 26.5 (CH_3); MS: *m/z* 447 [M^+]; Anal. Calcd. for $C_{20}H_{25}N_5O_3S_2$ (447.58): C, 53.67; H, 5.63; N, 15.65; Found: C, 53.45; H, 5.75; N, 15.55.

3-Acetyl-1-[4-(pyrimidin-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.7]dodec-2-ene (Vh): Yield: 65%; m.p.: 169-171 °C; IR (KBr) ν_{max}: cm^{-1} 3366, 3360 (NH's), 1692 (CH_3-C=O), 1626 (C=N), 1346, 1175 (SO_2); ^1H NMR ($CDCl_3$): δ 11.76 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 4H, Ar-H), 8.40 (d, 2H, pyrimidine), 6.81 (t, 1H, pyrimidine), 5.66 (s, 1H, NH triazole ring), 2.55 (*s*, 3H, $COCH_3$), 2.42-1.24 (m, 14H, cyclooctane); ^{13}C NMR ($CDCl_3$): *δ* 192.2 (C=O), 168.1-110.2 (Ar-C, C=N and pyrimidine-C), 91.8 (spiro-C), 41.8, 36.3, 27.1, 21.6 (cyclooctane-C), 26.6 (CH_3); MS: *m/z* 442 [M^+]; Anal. Calcd. for $C_{21}H_{26}N_6O_3S$ (442.54): C, 57.00; H, 5.92; N, 18.99; Found: C, 56.85; H, 6.05; N, 18.87.

3-Acetyl-8-methyl-1-[4-(thiazol-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.5]-dec-2-ene (Vi): Yield: 71%; m.p.: 186-188 °C; IR (KBr) ν_{max}: cm^{-1} 3374, 3355 (NH's), 1693 (CH_3-C=O), 1622 (C=N), 1346, 1175 (SO_2); ^1H NMR ($CDCl_3$): δ 11.70 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 4H, Ar-H), 8.71 (d, 1H, thiazole), 6.65 (d, 1H, thiazole), 5.64 (s, 1H, NH triazole ring), 2.54 (*s*, 3H, $COCH_3$), 2.07-1.14 (m, 8H, cyclohexane), 0.98 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): *δ* 189.9 (C=O), 168.2-116.5 (Ar-C, C=N and thiazole-C), 34.9, 27.8, 22.4 (cyclohexane-C), 90.9 (spiro-C), 31.4 (CH_3 at cyclohexane ring) 26.5 (CH_3); MS: *m/z* 433 [M^+]; Anal. Calcd. for $C_{19}H_{23}N_5O_3S_2$ (433.55): C, 52.64; H, 5.35; N, 16.15; Found: C, 52.85; H, 5.20; N, 16.28.

3-Acetyl-8-*tert*-butyl-1-[4-(thiazol-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro-[4.5]dec-2-ene (Vj): Yield: 69%; m.p.: 199-201 °C; IR (KBr) ν_{max}: cm^{-1} 3370, 3353, (NH's), 1695 (CH_3-C=O) 1622 (C=N), 1346, 1175 (SO_2); ^1H NMR ($CDCl_3$): δ 11.69 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 4H, Ar-H), 8.68 (d, 1H, thiazole), 6.63 (d, 1H, thiazole), 5.57 (s, 1H, NH triazole ring), 2.57 (*s*, 3H, $COCH_3$), 2.05-1.13 (m, 8H, cyclohexane), 0.92 (9H, s, $(CH_3)_3C$); ^{13}C NMR ($CDCl_3$): *δ* 189.9 (C=O), 168.2-116.5 (Ar-C, C=N and thiazole-C), 90.8 (spiro-C), 35.2, 27.9, 22.6 (cyclohexane-C), 46.9, 35.7, 32.4, 24.0 (*tert*-butyl carbons), 26.4 (CH_3); MS: *m/z* 470 [M^+]; Anal. Calcd. for $C_{23}H_{30}N_6O_3S$ (470.60): C, 58.70; H, 6.43; N, 17.86; Found: C, 58.55; H, 6.31; N, 17.95.

3-Benzoyl-1-[4-(thiazol-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.4]non-2-ene (VIa): Yield: 66%; m.p.: 219-221 ^{o}C; IR (KBr) ν_{max}: cm^{-1} 3368, 3345 (NH's), 1675 (Ph-C=O), 1596 (C=N), 1346, 1175 (SO_2); ^{1}H NMR ($CDCl_3$): δ 11.56 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 9H, Ar-H), 8.74 (d, 1H, thiazole), 6.60 (d, 1H, thiazole), 5.63 (s, 1H, NH triazole ring), 89.8 (spiro-C), 2.37-1.60 (m, 8H, cyclopentane); ^{13}C NMR ($CDCl_3$): *δ* 185.2 (C=O), 167.9-116.2 (Ar-C, C=N and thiazole-C), 34.6, 24.7 (cyclopentane-C); MS: *m/z* 467 [M$^+$]; Anal. Calcd. for $C_{22}H_{21}N_5O_3S_2$ (467.57): C, 56.51; H, 4.53; N, 14.98; Found: C, 56.25; H, 4.40; N, 15.10.

3-Benzoyl-1-[4-(pyrimidin-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.4]non-2-ene (VIb): Yield: 67%; m.p.: 236-238 ^{o}C; IR (KBr) ν_{max}: cm^{-1} 3371, 3347 (NH's), 1676 (Ph-C=O), 1597 (C=N), 1346, 1175 (SO_2); ^{1}H NMR ($CDCl_3$): δ 11.58 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 9H, Ar-H), 8.44 (d, 2H, pyrimidine), 6.81 (t, 1H, pyrimidine), 5.58 (s, 1H, NH triazole ring), 2.37-1.60 (m, 8H, cyclopentane); ^{13}C NMR ($CDCl_3$): *δ* 185.5 (C=O), 168.1-110.2 (Ar-C, C=N and pyrimidine-C), 89.9 (spiro-C), 34.8, 24.7 (cyclopentane-C); MS: *m/z* 462 [M$^+$]; Anal. Calcd. for $C_{23}H_{22}N_6O_3S$ (462.53): C, 59.73; H, 4.79; N, 18.17; Found: C, 59.55; H, 4.95; N, 18.05.

3-(2-Furoyl)-1-[4-(thiazol-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.4]non-2-ene (VIIa): Yield: 70%; m.p.: 197-199 ^{o}C; IR (KBr) ν_{max}: cm^{-1} 3375, 3355 (NH's), 1665 (Ar-C=O), 1616 (C=N), 1346, 1175 (SO_2); ^{1}H NMR ($CDCl_3$): δ 11.63 (*s*, 1H, SO_2NH), 78.27-7.06 (*m*, 7H, Ar-H), 8.66 (d, 1H, thiazole), 6.58 (d, 1H, thiazole), 5.65 (s, 1H, NH triazole ring), 2.37-1.60 (m, 8H, cyclopentane); ^{13}C NMR ($CDCl_3$): *δ* 173.6 (C=O), 168.2-116.4 (Ar-C, C=N and thiazole-C), 90.6 (spiro-C), 34.7, 24.6 (cyclopentane-C); MS: *m/z* 457[M$^+$]; Anal. Calcd. for $C_{20}H_{19}N_5O_4S_2$ (457.53): C, 52.50; H, 4.19; N, 15.31; Found: C, 52.67; H, 4.06; N, 15.43.

3-(2-Furoyl)-1-[4-(thiazol-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.5]dec-2-ene (VIIc): Yield: 71%; m.p.: 226-228 ^{o}C; IR (KBr) ν_{max}: cm^{-1} 3377, 3352 (NH's), 1666 (Ar-C=O), 1615 (C=N), 1346, 1175 (SO_2); ^{1}H NMR ($CDCl_3$): δ 11.64 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 7H, Ar-H), 8.67 (d, 1H, thiazole), 6.56 (d, 1H, thiazole), 5.60 (s, 1H, NH triazole ring), 2.05-1.12 (m, 10H, cyclohexane); ^{13}C NMR ($CDCl_3$): *δ* 173.5 (C=O), 168.2-116.5 (Ar-C, C=N and thiazole-C), 90.4 (spiro-C), 36.2, 25.1, 23.2 (cyclohexane-C); MS: *m/z* 471 [M$^+$]; Anal. Calcd. for $C_{21}H_{21}N_5O_4S_2$ (471.56): C, 53.49; H, 4.49; N, 14.85; Found: C, 53.35; H, 4.55; N, 14.73.

3-(2-Thenoyl)-1-[4-(thiazol-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.4]non-2-ene (VIIIa): Yield: 72%; m.p.: 218-220 ^{o}C; IR (KBr) ν_{max}: cm^{-1} 3365, 3345 (NH's), 1665 (Ar- C=O), 1612 (C=N), 1346, 1175 (SO_2); ^{1}H NMR ($CDCl_3$): δ 11.66, (s, 1H, SO_2NH), 8.27-7.06 (*m*, 7H, Ar-H), 8.70 (d, 1H, thiazole), 6.63 (d, 1H, thiazole), 5.63 (s, 1H, NH triazole ring), 2.35-1.58 (m, 8H, cyclopentane); ^{13}C NMR ($CDCl_3$): *δ* 174.6 (C=O), 168.1-116.2 (Ar-C, C=N and thiazole-C), 88.8 (spiro-C), 34.8, 24.9 (cyclopentane-C); MS: *m/z* 473[M$^+$]; Anal. Calcd. for $C_{20}H_{19}N_5O_3S_2$ (473.60): C, 50.72; H, 4.04; N, 14.79; Found: C, 50.90; H, 3.90; N, 14.65.

3-(2-Thenoyl)-1-[4-(pyrimidin-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.5]-dec-2-ene (VIIId): Yield: 73%; m.p.: 198-200 ^{o}C; IR (KBr) ν_{max}: cm^{-1} 3365, 3346 (NH's), 1660 (Ar-C=O), 1610 (C=N), 1346, 1175 (SO_2); ^{1}H NMR ($CDCl_3$): δ 11.59 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 7H, Ar-H), 8.38 (d, 2H, pyrimidine), 6.81 (t, 1H, pyrimidine), 5.61 (s, 1H, NH triazole ring), 2.04-1.13 (m, 10H, cyclohexane); ^{13}C NMR ($CDCl_3$): *δ* 174.8 (C=O), 168.4-110.5 (Ar-C, C=N and pyrimidine-C), 88.9 (spiro-C),

36.1, 25.3, 23.1 (cyclohexane-C); MS: *m/z* 482 [M^+]; Anal. Calcd. for $C_{22}H_{22}N_6O_3S_2$ (482.59): C, 54.76; H, 4.60; N, 17.41; Found: C, 53.60; H, 4.05; N, 19.35.

3-(2-Naphthoyl)-1-[4-(thiazol-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.4]non-2-ene (IXa): Yield: 61%; m.p.: 212-213 ^oC; IR (KBr) ν_{max}: cm^{-1} 3369, 3350 (NH), 1606 (Ar-C=O), 1608 (C=N), 1346, 1175 (SO_2); ^1H NMR ($CDCl_3$): δ 11.66 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 11H, Ar-H), 8.76 (d, 1H, thiazole), 6.66 (d, 1H, thiazole), 5.53 (s, 1H, NH triazole ring), 2.37-1.60 (m, 8H, cyclopentane); ^{13}C NMR ($CDCl_3$): δ 185.4 (C=O), 168.0-116.2 (Ar-C, C=N and thiazole-C), 89.8 (spiro-C), 34.9, 24.9 (cyclopentane-C); MS: *m/z* 517 [M^+]; Anal. Calcd. for $C_{26}H_{23}N_5O_3S_2$ (517.63): C, 60.33; H, 4.48; N, 13.53; Found: C, 60.15; H, 4.60; N, 13.65.

3-(2-Naphthoyl)-1-[4-(pyrimidin-2-yl-sulfonyl)phenyl]-1,2,4-triazaspiro[4.4]-non-2-ene (IXb): Yield: 62%; m.p.: 165-167 ^oC; IR (KBr) ν_{max}: cm^{-1} 3367, 3355 (NH's), 1655 (Ar-C=O), 1605 (C=N), 1346, 1175 (SO_2); ^1H NMR ($CDCl_3$): δ 11.67 (*s*, 1H, SO_2NH), 8.27-7.06 (*m*, 11H, Ar-H), 8.46 (d, 2H, pyrimidine), 6.85 (t, 1H, pyrimidine), 5.55 (s, 1H, NH triazole ring), 2.34-1.51 (m, 8H, cyclopentane); ^{13}C NMR ($CDCl_3$): δ 185.6 (C=O), 168.2-110.4 (Ar-C, C=N and pyrimidine-C), 89.7 (spiro-C), 34.8, 24.7 (cyclopentane-C); MS: *m/z* 512 [M^+]; Anal. Calcd. for $C_{27}H_{24}N_6O_3S$ (512.59): C, 63.27; H, 4.72; N, 16.40; Found: C, 63.40; H, 4.62; N, 16.28.

4. CONCLUSION

New series of novel functionalized spiro1,2,4-triazols **V-IXa-j** bearing sulfonamide moiety were synthesized upon the treatment of amidrazones **Va-j** with cyclic ketones in refluxing dioxane and evaluated for their in vitro antibacterial, and antifungal activities. From the screening results, it found to possess various antimicrobial activities towards all the microorganisms tested. The results confirm that, the antimicrobial activity is strongly dependent on the nature of the substituents on triazole ring.

5. ACKNOWLEDGMENTS

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KINETICS OF REACTION BETWEEN MALACHITE GREEN AND HYDROXYL ION IN THE PRESENCE OF REDUCING SUGARS.

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Abstract

The reaction was studied via pseudo first order kinetics using uv-1800 Shimadzu spectrophotometer with a thermostated cell compartment and interfaced with a computer . The reaction showed first order with respect to malachite green and sugar and hydroxyl ion concentrations. However, the reaction was independent of ionic strength and showed no dependence on salt effect, indicating an inner sphere mechanism for the reaction. There was no polymerization of the reaction mixture with acrylonitrile, indicating absence of radicals in the course of the reaction. Michaelis-Menten plot indicated the presence of a reaction intermediate in the rate determining step. The activation parameters of the reaction have been calculated and products were elucidated by FTIR spectroscopy. The stoichiometry of the reaction is 1:1. A mechanism consistent with the above facts has been suggested.

Keywords: Malachite green(MG), sodium hydroxide, Glucose, Fructose, Xylose.

Introduction

Malachite green is a triphenylmethane dye which has wide range of industrial applications, most especially in aquaculture production because of its antibacterial, antifungal and antiparasitic properties(1) . However, they are toxic to human as they have been confirmed to be mutagenic and carcinogenic (2). Owing to its hazardous effect, several researches have been reported on the best ways to remove it in water or industrial effluents mainly by biosorption(3,4,5,6). It has been established that wood fibre of phoenix tree(*Firmiana Sinplex*) is an effective adsorbent for malachite green(7).

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Fading of malachite green(MG) in the presence of hydroxyl ion has been attributed to the hydroxide ion attack on the central C atom of the planar ring system of malachite green, thereby destroying its conjugation orientation.It has been found that sugars such as dextrose, lactose and sucrose increases the rate of fading of dyes in general (8). Hitterto, there has been no report on the kinetics and mechanism of alkaline malachite green fading in sugars. However, few or scanty investigations have been reported on the kinetics of alkaline malachite green fading in alcohols and in the presence of surfactants(9,10).

Hence the need to study the effect of some reducing sugars on the alkaline fading of malachite green with the view to determining the mechanism via a vis the mechanism earlier proposed for the oxidation of sugars in sodium hydroxide medium(11).

Materials and methods

Analar grade malachite green, NaOH, KOH, glucose, fructose and xylose were used and the solutions were prepared in doubly distilled water.

The reaction products were characterized by FTIR and the reaction showed no presence of radicals as there were no polymerization on addition of acrylonitrile to the reaction mixture.

Spectral Measurement

λmax of dilute solution of malachite green was obtained at 617 nm using a uv-1800 Shimadzu spectrophotometer.

Kinetic Measurements

The reactions were performed under pseudo-first order conditions by maintaining a large excess (x10 or greater) of sugar over malachite green. The kinetic data were obtained by monitoring change in absorbance of malachite green at absorption maximum as a function of

time with a UV- 1800 Shimadzu spectrophotometer connected to a thermostated cell compartment and interfaced with a computer. Appropriate quantities of the solution of sugar, potassium nitrate, sodium hydroxide and malachite green were measured into the cuvette. Consequently, the reaction was kick started after adding requisite volume of the malachite green solution (All stock solutions were kept in the water bath for 30 minutes before the kinetic runs). The kinetic data were obtained via pseudo- first order condition with the concentration of the sugar in large excess compared with the oxidant concentration. The pseudo- first order rate constant (k_{obs}) were calculated.

Results and Discussion

Stoichiometry and product analysis

The stoichiometry of the reaction was determined by spectroscopic titration. Absorbances of solutions containing various concentrations of sugar within the range 1.00×10^{-2} to 6.00×10^{-2} and a constant $[MG]_o$ of 1.00×10^{-3} and constant $[NaOH]_o$ of 6.00×10^{-2} and $[KNO_3]_o$ of 0.25M were measured at 710nm after the reaction had gone to completion. The stoichiometry was evaluated from the plot of absorbance vs [sugar] curve and was found to be 1:1.

Effect of [MG] on the observed rate constant

A plot of In k_{obs} vs In[MG] gave a slope equal to 1, indicating a first order dependence. The reaction showed an increase in rate constant with increase in [MG] as shown in fig.1.

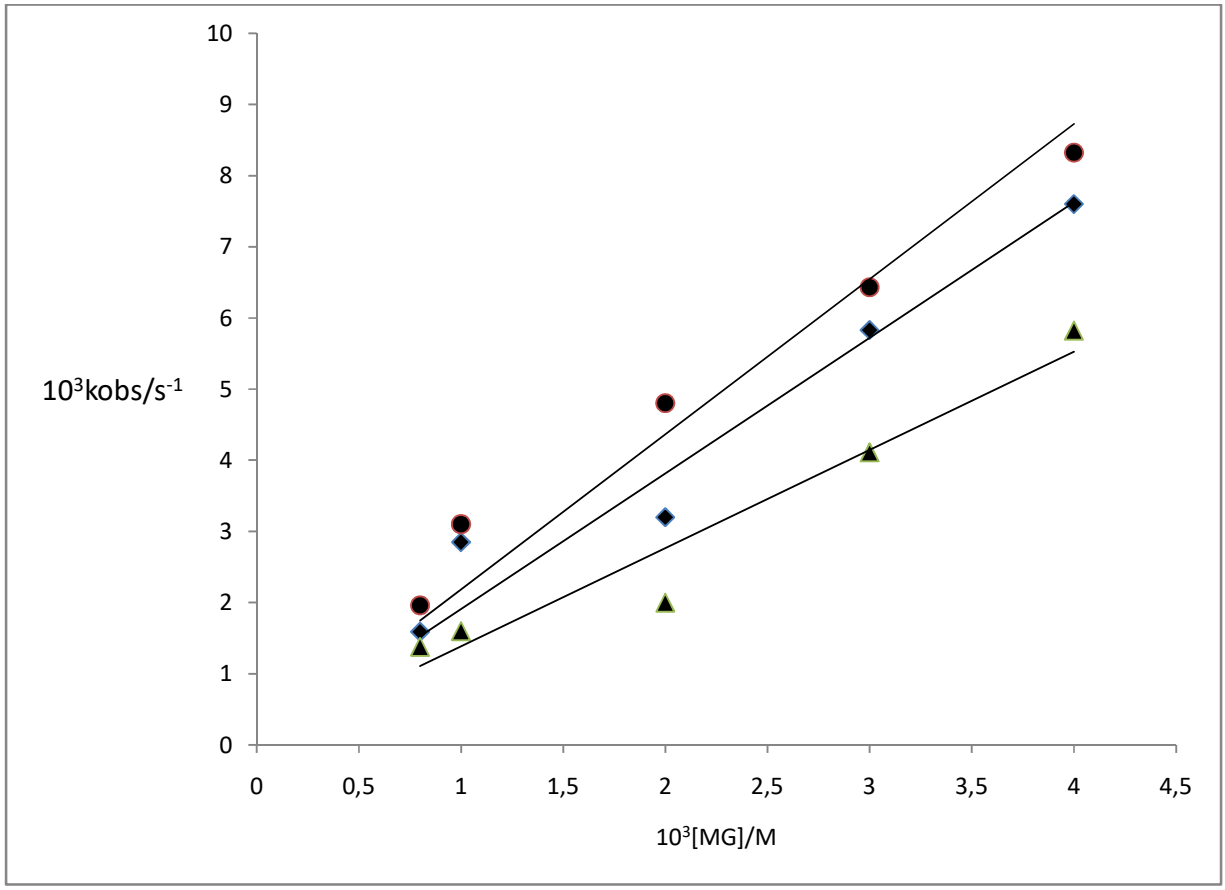


Fig. 1: Plot of k_{obs} vs [MG]

(● Fructose ■ Glucose ▲ Xylose)

Effect of [sugar] on the observed rate constant

As the concentration of sugars increases, there is increase in the rate constants as shown in table 1. The plot of In k_{obs} vs In[sugar] shows that the reaction increases with increase in sugar concentrations, indicating that the complex formation took place between sugar and malachite green and the slope of this plot is unity, implying a first order dependence on [sugar]. The plot of $1/k_{obs}$ vs 1/[s] gave an intercept , revealing the presence of an intermediate complex as shown in fig.2. Second order rate constants are 0.152 $M^{-1}S^{-1}$(Glucose), 0.138 $M^{-1}S^{-1}$ (Fructose), 0.125 $M^{-1}S^{-1}$ (Xylose).

Table 1: Effect of [sugar] on reaction rate

10^2[S]/M	$10^3k_{obs}/s^{-1}$		
	Glucose	Fructose	Xylose
1.00	1.81	2.10	1.73
2.00	3.60	3.40	2.81
3.00	5.12	4.75	3.62
4.00	6.84	6.02	4.80
6.00	8.10	7.41	6.51

[MG] 1.00 x 10^{-3}M [OH^-] 6.00 X 10^{-2}M I= 0.25M T=298K

Effect of [OH^-] on the observed rate constant

Increase in [OH^-] resulted into increased reaction rate as shown in table 2 . The slope of a plot of Ink_{obs} vs In[OH^-] also showed first order dependence.

Table 2: Effect of [OH-] on reaction rate

10^2[OH^-]/M	$10^3k_{obs}/s^{-1}$		
	Glucose	Fructose	Xylose
1.00	1.98	1.81	2.20
2.00	3.14	2.30	3.00
3.00	4.22	3.82	4.21
4.00	5.05	4.85	5.30
5.00	6.70	6.25	6.00
6.00	8.10	7.41	6.91

[MG] 1.00 X 10^{-3}M [S] 2.00 X 10^{-2}M I= 0.25M T= 298K

Effect of ionic strength on the observed rate constant

The reaction rate was independent of the ionic strength of the solution. A plot of Ink_{obs} vs $I^{1/2}$ gave a straight line with slope equal to zero as shown in fig.3, which implies the presence of a neutral molecule in the rate determining step.

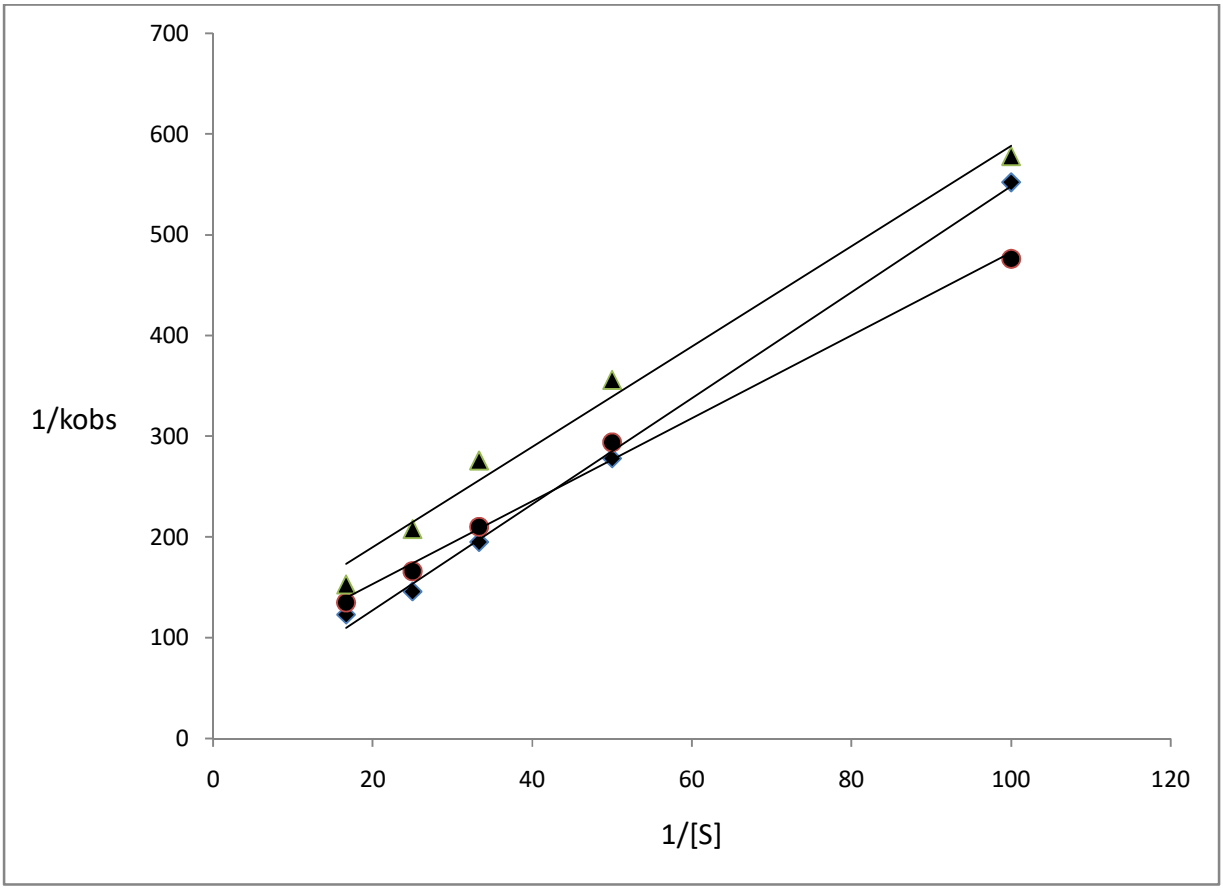


Fig.2: Plot of $1/k_{obs}$ vs 1/[S]

(● Fructose ■ Glucose ▲ Xylose)

Salt effect on the observed rate constant

The addition of KCl and $NaNO_3$ salts did not affect the reaction rate as shown in fig.4. This reveals an inner sphere mechanism for the reaction.

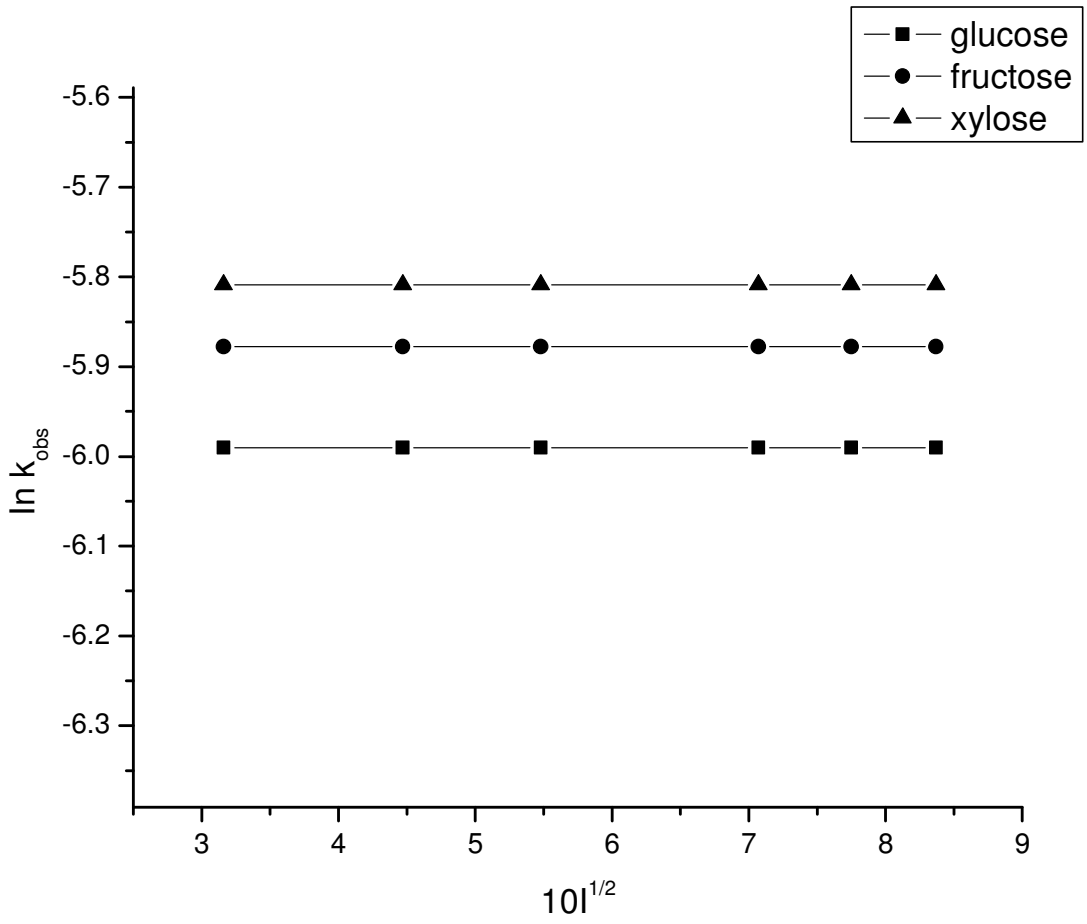


Fig. 3: Plot of Ink_{obs} vs $I^{1/2}$

Effect of temperature and determination of activated parameters

The effect of temperature on the observed rate constants were determined by varying the temperature within 298K- 308K and concentrations of all the reactants were constant: [MG] 1.00 X 10^{-3}, [sugar] 2.00 x 10^{-2}M, [OH^-] 6.00 x 10^{-2}M and I= 0.25M. The observed rates constants at different temperatures were determined and the values of activation parameters were calculated using the below relationship and shown in table 3. The result showed that the rate of reaction

increases with increase in temperature as this is evident by the high negative values of entropies of activation. Moreover, nearly constant values of Gibbs' free energies of activation suggests a similar mechanism for all the sugars.

Table 3: Activation parameters

Sugar	Ea(kJmol^{-1})	$\Delta H^{\#}$(kJmol^{-1})	$\Delta S^{\#}$(kJK^{-1}mol^{-1})	$\Delta G^{\#}$(kJmol^{-1})
Glucose	23.52	21.04	-0.182	75.28
Fructose	25.12	22.64	-0.177	75.38
Xylose	31.34	28.86	-0.156	75.35

[MG] 1.00 x 10^{-3}M [sugar] 2.00 10^{-2}M [OH^-] 6.00 X 10^{-2}M I= 0.25M T=298K

$$\log k = \log A - \frac{E_a}{2.303RT}$$

$$\ln\left(\frac{k}{T}\right) = \frac{-\Delta H^{\#}}{RT} + \ln\left(\frac{k^{/}}{h}\right) + \left(\frac{\Delta S^{\#}}{R}\right)$$

$$\ln\left(\frac{k^{/}}{h}\right) = 23.76$$

$\Delta G^{\#} = \Delta H^{\#} - T\Delta S^{\#}$

k = Rate constant

T= Temperature

$\Delta H^{\#}$= Enthalpy of activation

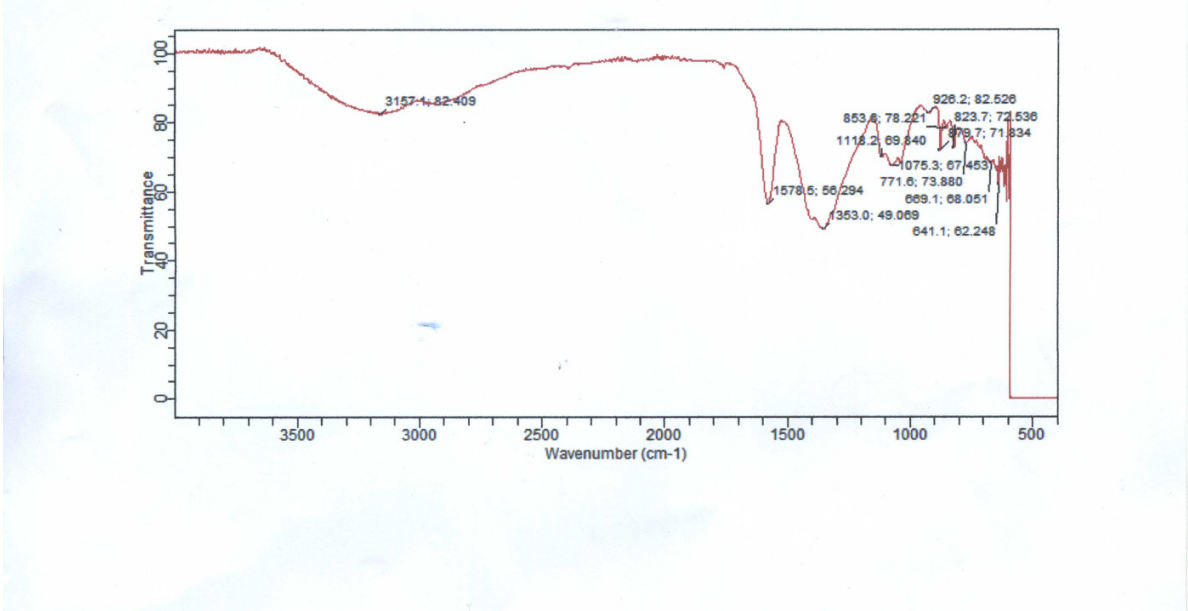
$\Delta S^{\#}$= Entropy of activation

$\Delta G^{\#}$ = Free Gibb's energy of activation

R= Molar gas constant

$k^{/}$= Boltzmann's constant

h= Plank's constant



FTIR Spectrum of the product.

Mechanism and Rate Law:

The non-dependence of the oxidation reaction on ionic strength indicates the presence of a neutral molecule in the rate determining step. Same values of $\Delta G^{\#}$ indicates same mechanism for the reaction and negative values of $\Delta S^{\#}$ revealed entropy decrease upon achieving the transition state, which often indicate an associative mechanism and it shows that the reaction occurred between ions of similar charges. The FTIR spectrum of the product showed very broad –OH stretching at 3400-2400 cm^{-1}, C=O stretching at 1730-1700 cm^{-1} and C-O stretching between 1320-1210 cm^{-1}which can be attributed to the presence of carboxylic acid. Michaelis-Menten plot showed the presence of an intermediate complex. Relatively low values of $\Delta H^{\#}$ revealed the absence of high energy free radicals.

Considering the above results, the following reaction mechanism is hereby proposed. The alkaline fading of malachite green in the presence of sugars proceeds via the conversion of sugars to their corresponding enediols which of course is a fast step. Then the enediol reacts with malachite green via an outer sphere mechanism to give a complex (slow step) which in turn decomposes into products.

$$S + OH^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} enediol(E)$$

$$E + MG \xrightarrow{k_2(slow)} products$$

$$\frac{-d[MG]}{dt} = k_2[E][MG] \qquad (1)$$

$$\frac{d[E]}{dt} = k_1[S][OH^-] - k_{-1}[E] - k_2[E][MG] \qquad (2)$$

$$0 = k_1[S][OH^-] - k_{-1}[E] - k_2[E][MG]$$

$$k_1[S][OH^-] = k_{-1}[E] + k_2[E][MG]$$

$$[E] = \frac{k_1[S][OH^-]}{k_{-1} + k_2[MG]} \qquad (3)$$

From equations (1) *and* (3)

$$\frac{-d[MG]}{dt} = \frac{k_2 k_1[S][OH^-][MG]}{k_{-1} + k_2[MG]} \qquad (4)$$

Limiting condition

If k_{-1} *is far greater than* $k_2[MG]$. *Then eq.* 4 *becomes*

$$\frac{-d[MG]}{dt} = K_1 k_2[S][OH^-][MG]$$

Conclusion

The research work showed that the mechanism of alkaline malachite green fading in reducing sugar is similar to that of the oxidation of sugars in alkaline medium. The reaction product as shown by the FTIR spectrum revealed the presence of carboxylic acid. This implies that sugars are been oxidized to their corresponding acids by malachite green and malachite green was decolorized via reduction process. However, in the absence of sugar, hydroxyl ion decolorizes malachite green by attacking the central C atom of the planar ring system of malachite green, thereby destroying its conjugation orientation which could have resulted in the production of carbinol.

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CHEMICAL CONSTITUENTS AND PHYTOCHEMICAL STUDIES OF EPAZOTE (*Chenopodium ambrosioides*) LINN GROWN IN NORTH CENTRAL NIGERIA

SCAN ME

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ABSTRACT

The essential oil from the leaves of C.ambrosioides of North Central Nigeria was obtained by hydro-distillation using Clevenger apparatus. The oil was analysed by a combination of GC and GCMS. Nineteen components amounting to 98.37% of the total oil were identified .The main constituents of the North Central Nigeria grown C.ambrosioides are 2-Carene(17.80%),2-bornene(14.79%),p-Cymene(12.93%),α-Terpinene(8.98%), Cyclohexene-4-methyl-3-(1-methylethylidene)(7.94%),α-Terpinolene(7.90%),γ-Terpinene (6.94%).The oil was found to be a yellow liquid, the obtained yield is 1.35%w/w based on the dry weight. The preliminary phytochemicalinvestigation showed that C.ambrosioides methanolic leaf extract contains some secondary metabolites such as Flavonoids, Terpenoids, Steroids, Alkaloids and Saponins. The result justified the use of C.ambrosioides in treating various infectious diseases.

KEYWORDS: *Volatile constituents, Phytochemical screening, Epazote, hydro distillation, Chenopodium ambrosioides.*

RESUMO

O óleo essencial de folhas de C. ambrosioides da parte Norte-Central da Nigéria foi obtido por hidrodestilação usando o aparelho de Clevenger. O óleo foi analisado com uma combinação de cromatografia gasosa e cromatografia gasosa e espectrometria de massa. Foram identificados 19 componentes correspondendo à 98,37% do total. Os constituintes principais de C. ambrosioides foram careno (17,80%), 2-borneno (14,79%), p-cimeno (12,93%), alfa-terpineno (7,90%), ciclohexeno-4-metil-3(1-metiletilideno) (7,94%), alfa-terpinoleno (7,90%) e gama-terpineno (6,94%). O óleo é um líquido amarelo e corresponde à 1,35% por peso do peso seco. Estudos preliminares fitoquímicos indicaram que as folhas contém flavonoides, terpenoides, sterois, alcalóides e saponinas e justificam o uso de C. ambrosioides no tratamento de doenças infecciosas.

PALAVRAS CHAVE: Chenopodium ambrosioides, Óleo essencial,Hidrodestilação, Estudos fitoquímicos, Constituintes voláteis

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INTRODUCTION

Higher and aromatic plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeast. Biologically active compounds from natural sources have always been of great interest to scientists [1].The essential oils known as volatile oils evaporate in contact with air and possess a pleasant fragrance. Chemically essential oils are very complex. They are found in many different species of plants of various families. All aromatic plants contain essential oils. Generally the oils are secreted in the oil glands [2]

According to WHO, about three quarter of the world population relies upon traditional remedies [mainly herbs] for the health care of its people. In fact plants are the oldest friends of mankind. They not only provide food and shelter but also serve the humanity to cure different ailments [3].

The plant family Chenopodiaceae is a large family comprising about 102 genera and 1400 species [4]. The genus Chenopodium include varieties of weedy herbs (more than 200 species) native to Europe, Asia and both North and South America [5]. Chenopodium ambrosioides Linn also called (Epazote, Mexican tea, Wormseed e.t.c) is a widespread species native to tropical America [6]. It is annual or short-lived perennial herbs that have been used for centuries as condiment, traditional purgative for intestinal worms and many other medicinal purposes [7-9]. The leaves and seeds are green when fresh. The plant is sometimes cultivated principally for its medicinal use in West Africa as purgative, for treating sores, edema and flavouring purposes [10]. They are employed for different ailments in Africa. The importance of Chenopodium species is due to their wide variety of medicinal properties [11].

C.ambrosioides is called Ewe-imi, Asin and Arunpale in some parts of Nigeria [12]. The whole plant leaves are used as anthelmintics, emollient and to treat rheumatism and tumour in parts of the country [13]. It has also been employed by empirical herbalist and healers against intestinal parasites (especially small tapeworms and roundworms) throughout Latin America; as well as West Indies [14]. As a result of its effect against intestinal parasites,traditional healersin North central Nigeria administer about 2-3 gm of the dried powdered leaves of C.ambrosioides mixed with pap to treat hookworm infection and hookworm inflammatory diseases, the decoction is also used in this region as wash for various skin diseases, to treat fever and as a fumigant against mosquitoes.

The biological activity of C.ambrosioides has been shown to affect viruses [15], bacteria [16], fungi [17], nematodes [18], and insects [19]. Furthermore the plant is traditionally and widely used as antiparasitic, anti-inflammatory and antibiotics which efficacy has been scientifically proven [20].

Chemical composition of the essential oil of C.ambrosioides from different parts of the world has been widely studied such as from Brazil [21], Cuba [22], Mexico [23], Cameroon [24], Nigeria [25], Rwanda [26], China [27,28], and India [29,30]. Ascaridole and Isoascaridole that were found in the Brazil grown C.ambrosioides [31] were absent in the North Central Nigeria grown C.ambrosioides, also α-Terpinene that constituted the major component in the Nigeria grown C.ambrosioides by[Gbolade et al,32] was just (8.98%) in the North Central Nigeria grown C.ambrosioides, 2-carene was found to be the major constituent.

Phytochemical are bioactive compounds found in plants they are non-nutritive compound (secondary metabolites) that contributed to flavour, colour [33, 34]. They are needed by plant for purpose such as disease, pathogen defence and control.The aim of this study was to identify the volatile constituents of the leaf oil of C.ambrosioides grown in North Central Nigeria by using modern GC/MS machine [SHIMADZU GCMS-QP2010 PLUS], as well as to find out the phytochemical components in the methanol extracts of C.ambrosioides grown in this region.

MATERIALS AND METHODS

Collection of plant leaves and identification

Fresh matured leaves of the plant were collected in May 2014 from a village in Anyigba of Kogi State, North Central Nigeria. They were identified by Dr Aina of the Department of Botany, Kogi State University, Anyigba. Voucher specimens are deposited in the herbarium of the Faculty of Bbiological Sciences, Kogi State University, Anyigba, Nigeria

Isolation of the volatile oil

The method employed for the extraction of the volatile oil of the leaf of Chenopodium ambrosioides was hydro distillation method according to European Pharmacopoeia (2008). Oils were collected and kept in the refrigerator without further treatment before GC-MS analysis.

Preparation of plant extracts of methanol

The collected fresh matured leaves of the plant of C.ambrosioides were air dried usually at room temperature in a well aerated room; the air dried leaves were grinded into coarse powdered using a Thomas Willey machine. The aqueous extract was prepared by soaking 200g

of the dried powdered samples in 400ml of methanol for 24 hrs. The extracts were filtered using Whatman filtered paper No 42 (125mm).

Screening of Phytochemical Components

To identify the phytochemical derivatives in the methanolic extract, standard phytochemicals screening was performed [35-37].Alkaloid test was performed by Meyer's tests, flavonoid and tannins by ferric chloride test, terpenoid and sterols by Salkowski's test, saponin by frothing test and anthraquinones by Borntrager's test.

Identification Test

The following tests were carried out so as to detect active chemical constituents such as alkaloids, tannins, flavonoids, anthraquinones, steroids, terpenoids and saponin.

Test for Alkaloids

1cm^3 of 1%HCl was added to 3cm^3 of the extract in a test tube. The extract was treated with a few drops of Meyer's reagent. A creamy white precipitate was observed indicating the presence of alkaloid.

Test for Tannins

About 0.5g of the dried powdered samples was boiled in20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue back colouration. There was no brownish green or blue colouration observed.

Test for Saponin

About 2g of the powdered samples was boiled in 20ml of distilled water in a water bath and filtered.10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously, then observed for the formation of emulsion.

Test for Flavonoids

About 0.5g of the extract was boiled with 5.0ml of distilled water and then filtered .To 2.0ml of this filtrate; a few drops of 10% ferric chloride solution were added. A greenish blue or violet colouration indicated the presence of a phenolic hydroxyl group.

Test for Terpenoids

5ml of the extract was mixed in2ml of chloroform, and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for Sterols

A few milligrams of the plant extract were dissolved in2ml of chloroform and then 2ml of concentrated H_2SO_4 was added from the side of the test tube. The test tube was shaken for a few minutes. Red colour development in the chloroform layer indicated the presence of sterols.

Test for Anthraquinones

An aliquot of 0.5g of the extract was boiled with 10ml of H_2SO_4 and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour change. There was no colour change.

2.2 Gas chromatography/mass spectrometry analysis

The chemical composition of the essential oil was analysed using GC/MS technique. The mass spectrometer was SHIMADZU GCMS-QP2010 Plus (Shimadzu Corporation, Japan) in the electron impact (EI) ionization mode (70eV) and HP-5MS (bonded 0.25μm) Capillary column (restek, Bellefonte, PA). Injector and Detector temperature were set at 250°C. The oven temperature was held at 60°C for 30minutes, then programmed to 240°C at rate of samples (1/100 in hexane v/v) of 1.0ml were injected automatically. The linear velocity of the column was 36.8cm/sec, each peak was then analysed and assigned a number in the order that it was detected. The identification of the components was based on comparison of their mass spectra with those of NIST library mass spectra database and literature.

Table 1. Volatile Constituents of the Leaf Oil of Chenopodium Ambrosioides.

S/N	RI	COMPONENT	% COMPOSTION
1	932	2-Bornene	14.79
2	948	IR-α-pinene	1.82
3	998	α-Terpinene	8.98
4	1001	2- Carene	17.8
5	1010	3,7,7-trimethyl-1,3,5-cycloheptatriene.	7.94
6	1023	4-methyl-3-(1-methylidene)-1-cyclo hexane	4.31
7	1042	ρcymene	12.93
8	1052	α-Terpinolene	7.90
9	1094	Nona-3,5-diene-2-ol.	1.88
10	1119	*m–Xylene*	4.61
11	1143	α-Terpineol	0.50
12	1179	γ-Terpinene	6.94
13	1195	Decyn-2-ol	1.94
14	1248	1,2:4,5:9,10-Triepoxydecane	1.88
15	1763	Z-10-pentadecen-1-ol	0.57
16	2007	9-Octadecenal	0.64
17	2085	16-Octadecenoic acid, methyl ester	1.44
18	2175	Oleic acid	1.14
19	2682	15-Tetracosenoic acid	0.36
TOTAL IDENTIFIED			98.37%

Note; RI Means Retention Index

RESULTS AND DISCUSSION

The hydro distillation of the leaf oil of *C.ambrosioides* yielded 1.35% w/w essential oil. Nineteencomponents amounting to 98.37% were identified in the leaf oil. The identified components, their retention index and percentage composition of each component is given in table1 above. Most of the components being monoterpenes and oxygenated monoterpenes. The major components found in the essential oil of *C.ambrosioides were* 2-*Carene (17.8%), 2-Bornene (14.79%), p-Cymene (12.93%), α-Terpinene (8.98%), Cyclohexene-4-methyl-3-(1-methylethylidene) (7.94%),α-Terpinolene (7.90%) and γ-Terpinene (6.94%).*

Constituents present in significant amount are *m-Xylene (4.61%), 3, 7, 7-Trimethyl-1, 3, 5-cycloheptatriene (4.01%), Decyn-2-ol (1.94%), Nona-3, 5-dien-2-ol, IR-α-Pinene (1.82%) e.t.c.* It is apparent that the main compounds characterizing the leaf oil are qualitatively and quantitatively different. It is also worth mentioning that compounds like *m-Xylene, nona-3,5-dien-2-ol, decyn-2-ol, 3,7,7-trimethyl-1,3,5-cycloheptatriene and 2-bornene* which are detected in the North Central Region of Nigeria samples have not been reported previously as part of the constituents of the volatile oil of *C.ambrosioides.*

The composition of the essential oil of *C.ambrosioides* has been the subject of several studies and data from literature shows that it has no constancy neither with respect to the

relative humidity, irradiance, photoperiod and the method of extraction, location, plant cultivation techniques, soil structure and climate heavily influence the composition and quality of essential oil. [38].These factors have been used to ascertain why exact specification of the component of essential oils is not acceptable.

The percentage constituents obtained for this sample shows that there is a marked difference in the earlier report given on *C.ambrosioides* leaf by different authors [39, 40& 32].The report shows that *C.ambrosioides*leaf was composed of α-terpinene as the major constituent. However, the major component of the North Central Region of Nigeria*C.ambrosioides* oilwas found to be 2-carene with17.8 percentage. Ascaridole was not detected in the North Central Nigeria oil of *C.ambrosioides.*

The phytochemical screening of the methanolic extract was carried out in order to analyse the presence of secondary metabolites such as flavonoids, alkaloids, terpenoids, sterols e.t.c] by utilizing standard methods [35-37]. Table 2 shows the results of the preliminary phytochemicals analysis. This study has revealed the presences of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as terpenoid, flavonoid, alkaloid, sterols, were present in *C.ambrosioides* leaves[Table: 2.] Plants rich in terpenoid have been reported to have anti inflammatory, antimalaria, antiviral, inhibition of cholesterol synthesis and antibacterial [41], while those rich in alkaloid are used in medicine to reduce headache and fever. These are attributed for antibacterial and analgesic properties [42]. Epidemiologic studies recommended that coronary heart diseases are opposed by dietary flavonoids. [42].

Table 2. Phytochemical Constituents of the Leaf of Chenopodium Ambrosioides.

Phytochemicals	**Test**	**Me**
Alkaloids	Meyer's Test	+
Tannins	Ferric Chloride Test	–
Saponin	Frothing Test	+
Flavonoids	Ferric Chloride test	+
Terpenoids	Salkowski Test	+
Anthraquinones	Borntrager's Test	–
Sterols	Salkowski Test	+

Key: (+) means Present; (-) means absent;Me meansMethanolic Extract

CONCLUSION

The results obtained in this study showed that *C.ambrosioides* possesess essential oil in the leaves of the plant and that their oil compositions were quantitatively and qualitatively different. This study represents the first to the best of my knowledge analysis of the leaves volatile constituents of *C.ambrosioides* by using a modern GCMS machine [SHIMADZU GCMS QP-2010 PLUS]. The phytochemical analysis showed the presence of effective biological compounds like alkaloids, steroids, flavonoids saponin and terpenoid in C.*ambrosioides* leaves thus providing knowledge of the phytochemical metabolites. Observation drawn from this experiment shows clearly that the leaves of *C.ambrosioides* contain phytochemical constituents. However the present investigation showed that the studied plant is a potential good source of traditional medicine and because it's a medicinal plant, it has commercial interest in both research institutes and pharmaceuticals company for the manufacture of new drugs in the treating of various diseases.

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VOLATILE OIL CONSTITUENTS OF THE LEAVES AND WOODS OF *Cedrela odorata* AND *Dalbergia latifolia* FROM SOUTH WEST NIGERIA.

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ABSTRACT

The essential oils from the leaves and woods of *Cedrela odorata* and *Dalbergia latifolia* were obtained by hydrodistillation and characterized by Gas Chromatography-Mass Spectrometry (GC-MS) method. A total of 10, 39, 72, and 6 compounds representing 97.63%, 99.16%, 95.13% and 99.83% were identified in *Cedrela odorata* leaf, wood, *Dalbergia latifolia* leaf and wood respectively. The major components in *Cedrela odorata* leaf are α-copaene (4.40%), α-santalene (4.57%), cubenol (8.71%), β-elemene (23.06%) and (-)-spathulenol (42.49%) while the wood was dominated by γ-eudesmol (8.84%), z-nerolidol (9.23%), β-bisabolol (10.95%), α-curcumene (12.31%) and α-cedrene (17.56%). The leaf essential oil of *Dalbergia latifolia* had in its composition majorly α-bergamotene (4.23%), (-)-spathulenol (4.49%), β-caryophyllene oxide (5.04%), α-selinene (4.89%), 1-heptariacotanol (5.71%) and heptacosane (6.31%) while the wood contained mainly methylcyclohexane (4.93%), hexanal (6.43%), m-xylene (16.71%) and p-xylene (58.8%).

Key words: Volatile oil, Sesquiterpenes, (-)-spathulenol, α-bergamotene, β-elemene

RESUMO

Os oleos essenciais de folhas e madeira de *Cedrela odorata* e *Dalbergia latifolia* foram obtidos atraves de hidrodestilação e caracterizados corn cromatografia gasosa e espectrometria de massa (GC-MS). Um total de 10, 39, 72 e 6 compostos representando 97.63%, 99.16%, 95.13% e 99.83% foram identificados em folhas de madeira de *Cedrela odorata* e folhas de madeira de *Dalbergia latifolia*, respectivamente. Os componentes majoritarios de folhas de *Cedrela odorata* são α-copaeno (4.40%), α-santaleno (4.57%), cubenol (8.71%). β-elemeno (23.06%) e (-) -espatulenol (42.49%). Na madeira prevaleceram γ-eudesmol (8.84%), z-nerolidol (9.23%), β-bisabolol (10.95%), α-curcumeno (12.31%) e α-cedreno (1756%). O óleo essencial de folhas de *Dalbergia latifolia* consistia principalmente de α-bergamoteno (4.23%), (-)-espatulenol (4.49%), óxido de β-carofileno (5.04%), α-selineno (4.89%), 1-heptariaoctanol (5,71%) e heptacosano (6.31%). Na madeira prevaleceram metilciclohexano (4.93%), hexanal (6.43%). m-xileno (16.71%) e p-xileno (58.8%).

PALAVRAS CHAVE: Óleos voláteis, sesquiterpenos, α-bergamoteno, β-elemeno, (-)-espatulenol

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INTRODUCTION

Essential oils or volatile oils are a complex mixture of compounds synthesized by living organisms and isolated from different parts of plants. Essential oils differ from plant to plant; though have a number of physical properties in common. A vast majority are known to have medicinal properties (1).

Cedrela odorata L. (Meliaceae) is commonly referred to as Spanish cedar wood, cigar-box tree or Brazilian mahogany. *C. odorata* is a monoecious, deciduous, and medium-sized to large tree up to 40 m tall that arrays from Mexico to South America and the West Indies (2). The tree is known for its red, rot-resistant wood that is used to make furniture, guitars (3) and cigar boxes. The repelling smell of the wood to insects makes it suitable for the making clothing chests and wardrobes. The wood from this tree is among the most sought-after in Latin America and elsewhere as it is resistant to attacks by fungi and insects, and it keeps a pleasant fragrance for many years (4).

The plant is used in traditional medicine; the decoctions of the bark are used as a remedy for wounds, fever, bronchitis, indigestion and other gastrointestinal ailments, vomiting, haermorrage (5). *C. odorata* essential oil has been shown to have anti-microbial activity (2) and the stem extract has been found to possess anti-oxidant activity (6). Kipassa et al., 2008 (7) isolated limonoids from the stem bark. The odour from the essential from *C. odorata* is in resemblance to the odour of cedar-wood and some species of Cyperus oils. It is used mainly for the perfumery of soaps, some types of sprays and disinfectant (8).

The chemical compositions of the essential oils of some parts of *C. odorata* have been investigated and generally, have sesquiterpenoids in abundance (2, 5, 6, 9, and 10). However, there are no comprehensive reports on the essential from the wood of *C. odorata*.

Dalbergia latifolia Roxb. commonly referred to as Indian rosewood or Bombay black belongs to the Fabaceae family. The plant is usually a single stemmed tree with a dome shaped crown of lush green foliage with characteristic smell (11). It is widely distributed in tropical and subtropical regions of Bihar, Bundelkhand and Central India (12). Many *Dalbergia* species are important timber trees which are valued for their decorative and fragrant wood, rich in aromatic oils (13). Traditionally, *D. latifolia* is reported to be used as stimulant, appetizer, anthelmintic, spasmogenic, and also in the cure of dyspepsia, diarrhea, cutaneous affections and leprosy (14). It is also regarded as brain tonic to the nervous system (15). Some parts of the tree are reported to have antioxidant scavenging potential and antibacterial activities (16)**.** Ethanol extracts of *D. latifolia* root neuropharmacological activity as nootropic and also having anxiolytic property, demonstrated in albino rats (17).

Though different phytochemical and pharmacological studies have been done on *D. latifolia*, there are no previous references in literature on the essential oils from parts of the plant.

This paper therefore, presents the comprehensive characterization of the essential oils from the leaves and woods of *C. odorata* as well as *D. latifolia* from South west Nigeria with a view to comparing any existing literature on the four oils.

EXPERIMENTAL

Fresh samples were cut in March, 2014 from mature trees growing at the Botanical garden, University of Ibadan. They were authenticated at the Department of Botany, University of Ibadan by Mr. Donatus and the voucher specimen was deposited at the Forest Research Institute of Nigeria (FRIN) Herbarium with Voucher Number, 110039 and 110038 for *Cedrela odorata* and *Dalbergia latifolia* respectively. The stem-bark was scrapped off the wood; the fresh wood and leaves were chopped into pieces, air-dried and crushed using a fast rotating grinding machine.

Essential oil isolation

The ground samples were subjected to the hydro-distillation method using an all glass Clevenger apparatus according to the *British pharmacopoeia* (18) method for 4 h. The oils were collected in hexane and kept in the refrigerator at 4 ^{o}C until analyses.

Gas chromatography/ Mass spectrometry Analysis

The composition of the essential oils were determined by a Gas Chromatography- Mass spectrometry (GC-MS) using a Agilent 7890N GC Agilent mass detector Triple Quad 7000A in EI mode at 70 eV (m/z range 40 – 600 amu) and an Agilent ChemStation data system. The GC column was equipped with an HP-5MS column (30 m × 250 μm × 0.25 μm) a split-split less injector heated at 200 ^{o}C and a flame ionization detector (FID) at 230 ^{o}C. The oven temperature was programmed as follows; initial temperature 40 ^{O}C for 5 mins increased 5 ^{O}C/min to 180 ^{O}C for 6 mins and then 10 ^{O}C/min to 280 ^{O}C for 12 mins. Helium was the carrier gas used at the flow rate of 1 mL/min. The injection volume was 2 μL (split ratio 1:20).

The components were identified by comparison of their mass spectra with NIST 1998 library data of the GC-MS system as well as by comparison of their retention indices (RI) with relevant literature data (19). The relative amount of each individual component of the essential oil was expressed as the percentage of the peak area relative to the total peak area. RI values of each component were determined relative to the retention times of a homologous n-alkane series with linear interpolation on the HP-5MS column.

RESULTS AND DISCUSSION

The chemical composition of the volatile oils of *C. odorata* leaf, *C. odorata* wood, *D. latifolia* leaf and *D. latifolia* wood are presented in **table 1**. The percentage yields of the oils (w/w) based on dry matter are 0.82%, 0.5%, 0.43% and 0.39% respectively. The four oils had different shades of yellow.

C. odorata leaf oil contained 10 compounds representing 97.63% of the entire oil with predominantly 85.21% sesquiterpenes with 51.65% oxygenated sesquiterpenes and 34.56% sesquiterpene hydrocarbon. The sesquiterpene hydrocarbons present are β-elemene (23.03%), α-bergamotene (4.09%), δ-cadinene (4.13%), α-humulene (2.28%0 while the oxygenated sesquiterpenes are (-)-spathulenol (42.49%), cubenol (8.71%) and germacrene-D-4-ol (0.45%). Monoterpene hydrocarbon in the oil represents 8.97% as characterized by α-santalene (4.57%) and α-copaene (4.4%). The results from this study when compared

with Asekun and Ekundayo, 1999 had some variations. Asekun and Ekundayo (10) characterized 26 compounds which amount to 70.6% of the oil identified with about 30% of the constituents unidentified. Their analyses also had sesquiterpenes as the abundant compound with α-santalene (9.5%), the dominant compound. Similar compounds to both studies are α-copaene, β-elemene, α-santalene, α-bergamotene, α-humulene, and δ-cadinene. Variations in qualitative and quantitative compositions observed from different studies are due to seasonal and maturity variation, geographical origin, genetic variation, growth stages and postharvest drying and storage (20).

The wood of *C.odorata* had 39 compounds amounting to 99.16% of the oil; with 54.92% sesquiterpene hydrocarbon and 37.40% oxygenated sesquiterpene. The remaining 6.84% of the characterized compounds are monoterpenes and non-terpenoid compounds. The abundant sesquiterpene hydrocarbon are α-cedrene 17.56%), α-curcumene (12.3%), α-bergamotene (5.63%), and, β-farnesene (3.89%) while oxygenated sesquiterpenes found in abundance are β-bisabolol (10.95%), z-nerolidol (9.23%), γ-eudesmol (8.84%, α-eudesmol (5.43%). Our study also revealed the presence of α-curcumene, β-bisabolol, juniper camphor, δ-cadinene as observed by Motl and Trka, 1973 (8).

It is notable that the variation in the composition of the leaf and wood essentials presumably because of different parts of the plants where the samples were procured. Common constituents to the oils are α-santalene, α-copaene, β-elemene, α-bergamotene, (-)-spathulenol, and α-humulene. Generally, the result of this study conveniently describes *C. odorata* as a sesquiterpene chemo type thus confirming previous qualitative and quantitative analyses (2, 7, 8, 10).

The leaf oil of *D.latifolia* contained 72 compounds representing 95.13% of the oil with sesquiterpenes hydrocarbons, oxygenated sesquiterpenes and non-terpene compounds. The sesquiterpene hydrocarbon constituted 31.10% of the oil while 12.35% are oxygenated sesquiterpenes and the remaining components non-terpene compounds. The most abundant compounds are α-bergamotene (4.23%, α-selinene (4.89%), β-caryophyllene oxide (5.04%, (-)-spathulenol (4.49%), heptacosane (6.31%, and 1-heptariacotanol (5.7%).

On the other hand, 6 non-terpene compounds were identified in the wood oil of *D. latifolia* representing 95.13% of the entire oil. They are toluene (6.43%), methylcyclohexane (4.93%), ethylbenzene (11.90%), p-xylene (58.80%), m-xylene (16.71%) and allyltetramethoxybenzene (1.06%).

Three of the four essential oils; *C. odorata* leaf, *C odorata* wood and *D. latifolia* leaf had in common (-)-spathulenol, α-bergamotene and β-elemene. These compounds have been found to possess different biological activities (21, 22, 23, 24, 25) and is a lead to explore the bioassay of the oils especially *D. latifolia* whose essential oils have never been the subject of literature discussion.

CONCLUSIONS

This paper provides a comprehensive characterization of the volatile oils from four different oils viz; *C. odorata* (leaf), *C. odorata* (wood), *D. latifolia* (leaf) and *D. latifolia* (wood). It thus gives a vivid comparison of the leaf oil of *C. odorat*a with previously presented studies in literature and has also reported for the first time the characterization of the essential oils of *C. odorata* wood, leaf and wood oils of *D. latifolia*. The identified compounds which have been reported in some literatures to possess different biological activities lend support for the use of the two studied plants in ethno medical practice for various purposes. It is therefore worthwhile to explore the various biological properties of the essential oils with a view to ascertain their bioactivity.

Table 1: Chemical Constituents of The Essential Oils of *Cedrela odorata* and *Dalbergia latifolia* Leaves and Wood.

RI	CONSTITUENTS	COL	COW	DLL	DLW
677	1-Methylpyrrole	-	-	3.22	-
752	3-Methylheptane	-	-	0.09	-
760	Ethylcyclopentane	-	-	0.07	-
781	Methylcyclohexane	0.8	-	2.53	-
794	Toluene	-	-	1.26	6.43
806	Hexanal	-	-	0.12	-
819	2, 4-dimethyl-1-heptane	-	-	0.15	-
842	(z)-1, 3-dimethylcyclohexane	-	-	0.08	-
852	4-methyloctane	-	-	0.13	-
877	Methylcyclohexane	-	-	-	4.93
880	Ethycyclohexane	-	-	0.06	-
887	3,5-dimethyloctane	-	-	0.12	-
893	Ethylbenzene	-	-	1.38	11.9
905	Heptenal	-	-	0.12	-
907	p-Xylene	-	0.22	-	58.8
907	m-Xylene	-	-	-	16.71
969	1-Octen-3-ol	-	-	0.31	-
974	Vinylhexanoate	-	-	0.14	-
986	2,6-dimethylnonane	-	-	0.08	-
1040	2-pentylfuran	-	-	0.51	-
1048	(z)-2-(2-pentenyl)-furan	-	-	0.06	-
1086	2,2,6-trimethylcyclohexanone	-	-	0.17	-
1104	Nonanal	-	-	0.88	-
1112	E-2-Nonenal	-	-	0.36	-
1120	(E,Z)-2,6-Nonadienal	-	-	0.26	-
1179	(+)-dihydrocarvone	-	-	0.07	-
1186	Safranal	-	-	0.06	-
1202	Decanal	-	-	0.12	-
1204	β-Cyclocitral	-	-	0.33	-
1211	α-Santalene	4.57	1.28	1.95	-
1221	α-Copaene	4.40	0.74	1.55	-
1231	Naphthalene	-	-	0.07	-
1281	Isoaromadendrene	-	0.40	-	-
1281	Isoaromadendrene epoxide	-	-	2.21	-
1286	Diepicedrene-1-oxide	-	-	0.90	-
1293	Aristolene epoxide	1.75	-	-	-
1303	2,6,6-trimethyl-1-Cyclohexene -1-acetaldehyde	-	-	0.17	-
1357	2-(2,6,6-trimethy-1-cyclohexen-1-yl) ethanol	-	-	0.66	-
1377	δ-Elemene	-	-	2.38	-

1393	2-butyl-1-octanol	-	-	0.15	-
1398	β-Elemene	23.06	2.08	3.96	-
1400	α-Longipinene	-	0.67	-	-
1403	α-Patchoulene	-	0.95	-	-
1406	(-)-aristolene	-	-	0.20	-
1410	α-Cedrene	-	17.56	-	-
1419	Isoledene	-	-	0.29	-
1424	E-1,10-dimethyl-E-9-decalinol	-	-	1.31	-
1425	Epi-β-Santalene	-	0.33	0.35	-
1429	E-α-Ionone	-	-	0.27	-
1430	α-Bergamotene	4.09	5.63	4.23	-
1431	Elocene	-	1.14	-	-
1440	β-Farnesene	-	3.89	-	-
1446	β-Sesquiphellandrene	-	2.43	-	-
1461	γ-Gurjunene	-	0.90	2.09	-
1462	Aromadendrene oxide-(1)	-	-	0.68	-
1469	δ-Cadinene	4.13	-	2.80	-
1470	β-Eudesmene	-	0.45	3.26	-
1474	α-Selinene	-	-	4.89	-
1480	δ-Guaiene	-	0.54	-	-
1489	Guaia-1(10),11-diene	-	-	0.72	-
1492	Isotridecylalcohol	-	-	0.16	-
1502	2-isopropenyl-4a,8-dimethyl-1,2,3 4,4a,5,6,7-octahydronapthalene	-	-	1.2	-
1507	β-Caryophyllene oxide	-	0.71	5.04	-
1512	Pentadecane	-	-	0.31	-
1522	Elemol	-	1.8	0.28	-
1524	α-Curcumene	-	12.31	1.40	-
1530	Ledol	-	0.41	0.84	-
1531	α-Bisabolene epoxide	-	-	0.14	-
1536	(-)-Spathulenol	42.49	1.12	4.49	-
1543	α-Cedrol	-	1.54	-	-
1547	α-Calacorene	-	-	0.2	-
1559	(E)-Longipinocarveol	-	0.52	-	-
1564	Z-Nerolidol	-	9.23	-	-
1574	Longiverbenone	-	0.22	-	-
1571	α-Caryophyllene	-	-	0.94	-
1579	α-Humulene	2.28	0.24	-	-
1580	Cubenol	8.71	-	-	-
1593	β-Eudesmol	-	-	1.59	-
1598	α-Eudesmol	-	5.43	-	-
1619	β-Bisabolol	-	10.95	-	-
1625	α-Bisabolol	-	2.73	-	-
1626	γ-Eudesmol	-	8.84	-	-
1645	6-isopentyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1H-napthalene-2-one		1.32	-	-
1647	Juniper Camphor	-	1.12	-	-
1660	Germacrene-D-4-ol	-	0.45	-	-

1680	Eudesm-7(11)-en-4-ol	-	-	3.87	-
1729	Murolan-3,9(11)-diene-10-peroxy	-	-	0.65	-
1739	Allyltetramethoxybenzene	-	-	-	1.06
1754	Hexahydrofarnesylacetone	-	-	2.19	-
1909	Labda-8(20),12,14-triene	-	0.50	-	-
1968	Hexadecanoic acid	-	0.25	-	-
1997	(9Z)-9,17-Octadecanal	-	0.26	-	-
2045	Phytol	-	-	0.65	-
2072	Cembrene	-	-	0.61	-
2075	Heptacosane	-	-	0.31	-
2228	(Z)-9-Octadecenamide	-	-	0.47	-
2271	Methyldehydroabietate	-	-	0.19	-
2375	Eicosylacetate	-	-	0.75	-
2704	Diisooctylpthalate	-	-	0.6	-
2705	Heptacosane	-	-	6.31	-
2788	p-Cresol,2,2-methylenebis[6-tertbutyl]-	-	5.30	-	-
2804	Octacosane	-	-	3.16	-
2944	Andrographolide	-	1.35	-	-
3942	1-Heptariacotanol	-	-	5.71	-
	Total	**97.63**	**99.16**	**95.13**	**99.83**

COL - *Cedrela odorata* Leaves; **COW - *Cedrela odorata* wood;** **DLL - *Dalbergia latifolia* leaves;**
DLW - *Dalbergia latifolia* wood. **RI – Retention Indices from present study.**

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CHEMICAL COMPOSITIONS AND ANTIMICROBIAL ACTIVITY OF THE LEAF ESSENTIAL OIL OF GOSSYPIUM HIRSUTUM

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Oyo State, Nigeria.**

ABSTRACT

The study was conducted to analyze the chemical constituent and to evaluate the antimicrobial properties of the leaf essential oil of Gossypium hirsutum. The oil was obtained by hydrodistillation and was analyzed by gas chromatography and gas chromatography –mass spectrometry (GC/GC-MS). This revealed the presence of fifty components accounting for - % of the total oil fraction. The leaf oil was dominated by patchoulane (14.70%), Ally 1-2, 6,6 – trimethyl bicyclo (3.1.1) heptane (5.95%). 1,7, 7 – trimethyl bicyclo (2.2.1) heptanes (5.95%) and 9- (1 – methylethylidene) bicyclo (6.1.0) nonane (5.95%). The oil displayed high antimicrobial potentials to some tested micro organisms.

KEYWORDS*: Gossypium hirsutum, patchoulane, aliphatic alcohol, antimicrobial potential.*

RESUMO

O estudo foi conduzido para analisar o constituinte químico e avaliar as propriedades antimicrobianas do óleo essencial de folha de Gossypium hirsutum. O óleo foi obtido por hidrodestilação e analisado por cromatografia gasosa e cromatografia gasosa-espectrometria de massa (GC / GC-MS). Isso revelou a presença de cinqüenta componentes representando -% da fração de óleo total. O óleo de folha foi dominado por patchoulane (14,70%), Ally 1-2, 6,6 - trimetilbiciclo (3.1.1) heptano (5,95%). 1,7, 7-trimetilbiciclo (2.2.1) heptanos (5,95%) e 9- (1-metiletilideno) biciclo (6.1.0) nonano (5,95%). O óleo apresentou potenciais antimicrobianos elevados para alguns microrganismos testados.

PALAVRAS CHAVE: *Gossypium hirsutum, patchoulene, álcool alifático, potencial antimicrobiano*

Introduction

Plants are one of the five main groups of living things, these groups are called kingdoms. The plant kingdom includes such living things as grasses, trees, ferns, bushes and flowers. About 260,000 species of plant exist on the earth (14) plant contains complex and powerful substances known as essential oils. These are aromatic liquid derived from shrubs, flowers, trees, roots, bushes, herbs and seeds (15). Medicinal plants form an aspect of cultural heritage of the human race and their use is still expanding. Plants with useful medicinal properties have been obtained from folklore records of many indigenous communities. Egyptian and Chinese priests and physicians since time immemorial had been using extracted oils from plant for healing and this makes essential oil the earliest known medicine (15). An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. It is also known as volatile oils, ethereal oils or aethrolea or simply as the “oil of’ the plant from which they were extracted. An oil is “essential” in the sense that it carries a distinctive scent or essence of the plant (16).

Gossypium hirsutum L. (family malvaceae) is a cotton specie native to Central America and the Caribbean. After being domesticated in the United States, this cotton species now provides over 90% of commercial cotton worldwide (13). In Nigeria, cotton seed provides raw materials for local spinning and weaving industry (4). It is believed to be an indigenous effective medicinal plant. The dried root bark of cotton contains gossypol, this compound may cause abortion – inducing effect (3). Gossypol is a polyphenol that may be isolated more easily from the bark (5). There is on going research to use gossypol as an alternative to vasectomy (7), the plant is known to

posses anti-malaria properties and it has demonstrated in vitro effects on cell regulation and antitumor activity against mammary carcinoma a cell lines (9, 18, 6).

When the chemical constitution of the essential oil of six species within three genera of the family malvaceae were investigated, the essential oil of Gossypium tursutum L. was found to contain 67.0% hydrocarbon and beta-bisabolol was the major oxygenated component 13.7% (1),also (12) reported the presence of fifty nine constituents in the oil aroma of Gossypium barbadense in which patchoulane (7.94%), Bicyclo (4.1.0) heptanes -3-cylopropy 1-7-hydroxymethyl (trans) (7.86%), Z-Nonadecatetraene (6.19%), alpha-patchoulane (6.19%), caryophyllene oxide (5.75%) and Bicyclo (7.2.0) undec-4-ene, 11, 11-trimethy 1-8-methylene (IR*, 4z, 95*) (3.50%) as the major constituents. The oil also has antibiotic potential (12, 13).

Materials and Methods

Plant Material

Leaves of Gossypium hursutum were collected from Oyo, south West, Nigeria. The plant was authenticated at the Forest Research Institute of Nigeria (FRIN) Ibadan, Nigeria. Extraction of the hursutum was air dried in a well ventilated place till when the moisture content reduced to a minimum suitable for grinding. The plant material was pulverized and used immediately. The crushed plant material (400g) was subjected to hydrodistillation for 3 hours using a Clevenger – type apparatus (11). The oil collected was stored in a vial at low temperature until the time of the analysis.

Multidimensional GC X GC-MS Analysis

Analysis of leaf essential oil of Gossypium barbadense was performed using multidimensional chromatogram coupled with gas chromatography, mass spectrometer (Shimadzu, Japan,) equipped with double capillary columns (2.50m X 0.25^{N}m I.d, 0.25^{N}mdf) that have different characteristics (non polar and polar) High purity helium was used as the carrier gas at a constant flow rate of 0.99ml/min. A total of 1Nl sample was injected (Split ratio 100:1) into GC X GC MS using AOC 20i auton injector for analysis. The initial temperature was set at 60^{0}C treated at a rate of 30^{0}C minute to 28^{0}C and held isothermally for 6 minutes. Ion source temperature for this analysis was set at 200^{0}C while the interface temperature was set at 250^{0}C solvent cut time was 3.0 minutes and the mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70eV as acquisition mass range from 40 – 700 a.m.N at 0.50cscan/S.

Antimicrobial Activities

The antibacterial activity of the leaf essential oil of Gossypium barbadense was measured against Grain-negative (Esherichia coli, klebsiellla pneumonae, salmonella typhimourion and proteins mirabilis) and Grain – positive (staphytlococcus aureus and streptococcus agalactiae) using a well diffusion method according to the National Committee for Clinical Laboratory Standard (13). Petri plates containing approximately 25-30ml of nutrient agar medium were swabbed using cotton applicator with 24 hours sub-cultured bacteria strains which were prepared in dilution to match the turbidity intensity of the MacFarland standard. Wells (6mm diameter) were punched in the agar

and filled 10^{N}L of the extract of different concentration (1000, 100 and 10Ngml^{-1}). The plates were incubated at 37^{0}C for 24 hours. The antibacterial activities were assessed by measuring the inhibition zone diameter (mm) around the well.

Results and Discussion

Exhaustive hydro distillation of the leaf of Gossypium hursutum gave a light yellow coloured oil with 0.4% V/W yield per 400g of dried leaf sample. The analysis of the leaf essential oil was carried out using GC X GC – MS and the percentage compositions are given in table 1. Fifty compounds were identified from the leaf essential oil amounting to –% of the oil. The leaf essential oil was dominated by patchoulane (14.70%) Allyl – 2, 6, 6 – trimethyl bicyclo (3.1.1) heptanes (5.95%), 1, 7, 7- Trimethyl bicyclo (2.2.1) heptanes (5.95%) and 9-(1-methyl-ethylidene) bicyclo (6.1.0) nonane (5.95%). The composition of the essential oil has similarity with the constituent of Gossypium barbadense reported by (12), patchovlane, been the compound with the highest percentage in Gossypium barbadense has the highest percentage in Gossypium hirsutum(14.70%). The aliphatic reported by (12) as part of the constituents of Gossypium barbadense are also in the present study even though it is quantitatively different (6 – Mehthyl-2-Vingl-5-hepten-01 (1.30%), 2- pentadecen-1-01 (1.30%) and Z – 10-pentadecen-1-01.

In addition, the oil displayed high antimicrobial activities to proteus micrabelis at 1,000, 100 and 10 Ngml^{-1}, Esherichia coli at 1,000, 100 and 10Ngml^{-1}, staphylococcus aureus at 1,000, 100 and 10Ngml^{-1} and klebsiell pneumonia at 1,000, 100 and 10Ngml^{-1} as shown in table 2. The oil also displayed low inhibitory activity against gram negative

salmonella typhimurium at all concentrations and this can antibiotics as shown in table 3.

Conclusion

The result obtained in this study showed that Gossypium hirustum possess essential oil and it has a great antibiotics potential and this confirm its use in traditional medicine as an antibiotics and anti-malaria.

Table I

Chemical Constituents of the essential oil of Gosspium Hirustum.

S/N	R.I	Compound	%Comp
1.	1061	2 (3H) – Furanone	2.00
2.	837	2 – Pentanethol	4.00
3.	935	Nitrohexane	2.00
4.	919	Pyruvic acid, Methylester oxime	2.00
5.	620	3 – Ethoxy-1-butene	0.58
6.	1273	3, 5 – Octanedione, 2, 2, 4, 7 – tetramethyl	0.58
7.	821	Pentanal, 2, 2 – dimethyl	0.58
8.	676	Tert – butylcarbinol	0.58
9.	729	Methyl -2-2methyoxyacrylate	0.58
10.	1515	Germacrene	0.2
11.	1221	Copane	0.4
12.	1440	Alpha – Amorphene	0.2

13.	1458	(Z, Z) alpha – Farnesene	0.2
14.		2, 6 – Bis (diazn) adamatane	0.32
15.	1050	2, 8 – Decadiyne	0.32
16.	1229	Ethanone-1-2-5 (methyl)-2 forylcycloprophyl	0.32
17.	2139	Benzene, 2-pheyl-2-propenyl) sulfonl oxacyclo tetradeca-4, 11-ddiyne	0.32
18.	1393	Patcheulane	14.70
19.	1148	1-Methyl-2-methylene-3, 5-divinyl cyclohexane	1.40
20.	1494	4,11, 11-Trimethyl-8-methylene bicyclohexane	1.40
21	1369	Cyclohexene, 4-180 propenyl-1-methoxymethyl	1.40
22.	1579	Alpha Humulene	0.63
23.	958	Ocimene	1.26
24.	976	Beta-ClS-Ocimene	1.26
25.		3-Methyl-4-(phenylthia)-2-prop-2-enyl-2, 5-dihydrothio phenel, 1-dioxide	0.57
26.		Lauryl pyridinium chloride	0.57
27.	1128	Pentanol-5- (methylene Cyclopropyl)	0.57
28.	1934	Z, Z, Z – 4, 6, 9 – Nonadecatriene	0.57
29.	1414	1, 2-Epoxy – 5, 9 – Cyclododecadiene	0.57
30	1187	Allyl – 2, 6, 6 – trimethyl bicoclo (3.1.1) heptanes	5.95
31.	1326	1, 7, 7 – trimethyl bicyclo(2.2.1) heptanes	5.95

32.	1242	9 - (1 – Methylethylidene) bicycle (6.1.0) nonane	5.95
33.	1066	1-(l-Methylene-2-propenyl) cyclopentanol	1.56
34.	1307	Bicyclo (4.1.0) heptanes-3-cyclopropy 1-7-hydroxymethyl trans	1.56
35.	1307	Bicuclo (4.1.0) heptanes-3-cycloprophy1-7-hydroxymethyl cis	1.56
36.	1078	1-(2 –Methylene cyclopropyl) cyclopentanol	1.56
37.	1143	Ethyhdene cyclo octane	1.56
38.	1196	Dihydrocarveol	1.30
39.	1169	6 – Methyl – 2-Vinyl-5-hepten-1-01	1.30
40.	1772	2 – Pentadecyn-1-01	1.30
41.	1292	Myrcenylacetate	1.30
42.	1079	Isothujol	1.30
43.	1968	Palmitic acid	3.27
44.	1272	Pelargic acid	1.09
45.	1769	Myristic acid	1.09
46.	2175	Cis-Oleic acid	4.00
47.	1763	Z-10-pentadecen-1-01	4.00
48.	2007	9 – Octadecenal	4.00
49.	1763	E-10-Pentadecen-1-01	4.00
50.	1293	10 – Undecenal	4.00
		Total	

Table 2

Zones of inhibition (mm) showing the antimicrobial activities of gram-negative and gram-positive bacteria.

Organism	**Escherichia Coil**	**Klebsiella Pneumonia**	**Salmonella Typhimouriu m**	**Proteus Mirabelis**	**Staphylococos**	**streptococcos**
Conc. ($Ngml^{-1}$)	1,000 100 10	1,000	1,000 100 10	1,000 100 10	1,000 100 10	1,000 100 10
Zone of inhibition	201710	151515	09 08 -	32 30 24	20 20 20	11 11 11

Keynote: - = no inhibition, 6-9mm = low inhibition, 10 – 15mm = moderate inhibition

And ≥ 15mm = high inhibition

+- = gram-negative bacteria

* = gram-positive bacteria

Table 3

Zones of inhibition (mm) showing the antimicrobial activities of antibiotics against gram positive and gram negative bacteria.

Organism/ antibiotics/ concentration	**Escherichia Coil**	**Klebsiella Pneumonia**	**Salmonella Typhimouriu m**	**Proteus Mirabelis**	**Staphylococus aureus**	**streptococcus agalactiae**
Nitrcoforantion (2Ng)	25	20	15	15	28	11
Ofloxacin (5Ng)	20	20	22	33	20	-
Gentamicin (10Ng)	24	15	18	16	-	-

Keynote: - Same as in table 2.

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SCAN ME

BOOKS / LIVROS

Enciclopédia dos minerais do Brasil abrange praticamente todas as substâncias minerais existentes em nosso território e suas localizações. Dividida em sete volumes, traz as seguintes temáticas: Elementos Nativos e Halogenetos; Sulfetos e Sulfossais; Óxidos e Hidróxidos; Carbonatos, Sulfatos e Combinações Orgânicas; Fosfatos, Arsenatos e Vanadatos; Silicatos.

Próximo lançamento:

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ENCICLOPÉDIA DOS MINERAIS DO BRASIL

CARBONATOS, SULFATOS E COMBINAÇÕES ORGÂNICAS

Paulo César Pereira das Neves
Daniel Atencio

Editora da ULBRA

Sumário

The four volumes (in Portuguese) are part of a monumental work that covers practically all the mineral species present in Brazil. The work consists of six volumes and four above have already been published.

The two authors have a vast professional experience.

According to the International Mineralogical Association 4893 mineral species have been validated world wide up to the present date. Of these, 894 species have been registered in Brazil. This monumental work represents the major compilation of minerals present in Brazil and is of extreme scientific importance, not only for Brazil.

Some fifty years ago, and Old American Indian Friend from New Mexico – The Land of Enchantment, told us that there are four things in Nature and the Universe that fascinate mankind with their beauty, radiance and magic. They are the ***stars,the flowers, the minerals and the sparkling eyes of beautiful women.***(Cf. L. G. Ionescu, South. Braz. J. Chem.,11(12), 22. 2003).

(The 4th volume of Enciclopédia dos minerais do Brasil. *with Carbonates, Sulfates, and Organic Compounds. is a considerable improvement of this monumental work written by Neves and Atencio. We congratulate the authors, and highly recommend this work to all persons interested in the Earth Sciences.)*

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De acordo com a ***International Mineralogical Association*** 4893 espécies minerais foram validadas até o presente à nível global. Destas, 894 espécies foram registradas no Brasil. A realização desta obra monumental que representa a maior compilação de minerais existentes no Brasil é de máxima importância para a ciência no nosso País.

Os dois autores possuem ampla experiência profissional.

Cínqüenta anos atrás, um Velho Indio Americano, nosso amigo do Novo México – Terra do Encanto, dizia que tem quatro coisas que são verdadeiramente bonitas e extraordinárias na Natureza e no Universo, que fascinam e encantam com a sua beleza, brilho e magia. São ***as estrelas, as flores, os minerais e os olhos cintilantes de mulheres bonitas.***
(Cf. ***L.G. Ionescu, South. Braz. J. Chem.,11(12)***, 22, 2003).

A presente obra com seu alto nível científico e altíssima qualidade gráfica nos traz uma das partes mais fascinantes do Universo Brasileiro.

O quarto volume da *Enciclopédia dos minerais do Brasil*, com Carbonatos, Sulfatos e Combinações Orgânicas, é um considerável incremento nesta monumental obra que está sendo escrita por Neves e Atencio. Congratulamo-nos com os autores e recomendamos este trabalho a todos aqueles que se interessam pelas Ciências da Terra.

Lavinel G. Ionescu, A.A., B.S., M.S., Ph.D. (Físico-Química/Astrofísica)

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