

DEDICATION

THIS WORK IS DEDICATED TO

MY SON: AWAD HASHIM

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I would like to thank Allah who gave me strength, health and patience to complete my study.

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ABSTRACT

Acacia sayal(Fabaceae), *Combretum hartmannianum* and *Terminalia laxiflora*(Combretaceae) are known for their medicinal uses in Africa and Asia. An ethnobotanical study of community-based segment of the women(100 women) for the three trees in Khartoum State - Sudan, with emphasis on three localities Khartoum, Khartoum North and Omdurman was carried out, the most common names of trees of *A. sayal*, *C. hartmannianum* and *T. laxiflora* used in these localities were: *A. sayal* was *Talh* (81%), while (19%) was *Makntosh*; *C. hartmannianum* was *Habeel* (100%) and *T. laxiflora* was *Sobage* (80%), *Darot* (12%) *Kolit* (8%), the common names of the fermented wood *Nikhra* of *A. sayal*, *C. hartmannianum* and *T. laxiflora* used in Khartoum state were: *Nikhra* (80%), *Nukhara* (10%) and *Guur* (10%). Analysis of 100 questionnaire showed that mostly married females (73%) use *A. sayal*, *C. hartmannianum* and *T. laxiflora* while only 27% single females used these plants. All the questionnaire responders agreed on the usage of the fermented wood as a cosmetic, i.e. *Dokhan* purpose. In this regard there were no differences in their response irrespective of differences in their age, jobs and level of education. *A. sayal* was found be used for cosmetic *Dokhan* purpose by 68% of the respondents, followed by 25% Combretaceae for *T. laxiflora* and 20% for *C. hartmannianum* the study also revealed that the trees, fermented wood, were also used as *Bakhour* by 50% of the respondents for *T. laxiflora* followed by 22% for *A. sayal* and 1% for *C. hartmannianum*. Other uses include mosquitoes repellent; fuel and fodder were also reported.

Fungi fermented heartwood *A. sayal*, *C. hartmannianum* and *T. laxiflora*, methanolic extracts percentage yielded where(2.99, 3.10 and 3.64) while the non fermented samples percentage yielded where (0.64, 0.92 and 2.27) respectively. All methanolic extracts were powder and fragrant, of dark and faint brown colors. Methanolic extracts of *Nikhra* of *T. laxiflora*, *C. hartmannianum* and *A. sayal* were fractioned using petroleum ether, ethyl acetate, chloroform and aqueous.

Nikhra fractions of *T. laxiflora* were 0.39, 1.68, 0.16 and 1.71%, while the non fermented were 0.20, 1.31, 0.05, and 0.71%, respectively. *Nikhra* fractions of *C. hartmannianum* were 0.36, 0.35, 0.49, and 1.27%, while its non fermented fractions were 0.27, 0.21, 0.09, and 0.35%, respectively. *Nikhra* fractions of *A.seyal* were 0.21, 0.97, 0.92, and 0.89%, while its non fermented fractions were 0.06, 0.36, 0.05, and 0.17% respectively.

Organoleptic survey of fragrance for a group of women questionnaires in different fractions of *A. seyal*, *C. hartmannianum* and *T. laxiflora* *Nikhra* showed that petroleum ether and ethyl acetate fractions of the three plants studied were most fragrant. Questionnaires revealed that *A. seyal* fragrance is preferred 53% followed by *T. laxiflora* 47% and *C. hartmannianum* is not an aromatic plant it is mostly, used for medicinal purposes (89%).

ANOVA statistic analysis for study ability of organic solvents (petroleum ether, ethyl acetate, chloroform and aqueous) to extract compounds from fermented and non fermented wood of *A. seyal*, *T. laxiflora* and *C. hartmannianum*, showed that weight of fractions of fermented wood of plants studied are bigger than non fermented wood of plants. On comparing f ratio and f table, f ratio is practically, bigger than f table. Hence fermentation and polarity of solvent have a clear impact on the weight of the extract.

The minimum inhibitory concentration (MIC) of the ethyl acetate fraction of *C. hartmannianum* against (*S. aureus*, *S. typhi* and *E.coli*) were (0.04, 0.04, 0.07) mg/ml respectively, while ethyl acetate fraction of *T. laxiflora* against (*S. aureus*, *S. typhi* and *E.coli*) were (0.04, 1.25, 0.005) mg/ml respectively and ethyl acetate fraction of *A. seyal* were (0.005, 0.04, 0.15) mg/ml respectively.

The MIC of the chloroform fraction of *C. hartmannianum* against *C. albicans* was 0.07 mg/ml, The MIC of both *A. seyal* and *T. laxiflora* was 0.15 mg/ml against *C. albicans*.

The MIC of chloroform fraction of *C. hartmannianum* against *A. flavus* and *A. niger* were (0.3, 0.04) mg/ml respectively, The MIC of the chloroform fraction of *T. laxiflora* against both *A. flavus* and *A. niger* was 0.6 mg/ml. The MIC of the chloroform fraction of *A. seyal* against both *A. flavus* and *A. niger* was 0.6 mg/ml

Fractions of ethyl acetate of the three plants were most active antioxidant by using the radical scavenging technique of DPPH we found antioxidant activity percentage of *A. seyal* (91 ± 0.02), *T. laxiflora* (90 ± 0.01), and *C. hartmannianum* (89 ± 0.01) with half minimum inhibitory concentration IC_{50} of *A. seyal* (0.482 ± 0.073), *T. laxiflora* (0.347 ± 0.0260), *C. hartmannianum* (0.460 ± 0.026) mg/ml. Fractions of the ethyl acetate of three plants which have most active antioxidant were so showed the highest phenolic compounds *A. seyal* (424.65) mg GAE/g, *T. laxiflora* (594.60) mg GAE/g, and *C. hartmannianum* (404.96) mg GAE/g.

All fractions of three plants proved to be non toxic against *A. salina* except ethyl acetate and chloroform fractions of *A. seyal* and chloroform fractions of *C. hartmannianum* which possessed slight toxicity.

Phytochemical screening showed that alkaloids, flavonoids, cardiac glycosides, saponins, tannin and triterpenoid / steroid were present in all fractions of *A. seyal*, *T. laxiflora* and *C. hartmannianum*. Triterpenoid / steroid were absent in chloroform fraction of *T. laxiflora*, and it was also absent in both ethyl acetate, chloroform. Triterpenoid / steroid were absent in petroleum ether fractions of *C. hartmannianum*.

Questionnaire showed that *Nikhra* fragrance was stronger than non fermented wood and by using (TLC) in three plants. Fragrance in the petroleum ether and, ethyl acetate fractions were also proved to be of different scents. Additionally, the ethyl acetate fraction especially that of *A. seyal* was fine textured

with golden yellow color. Petroleum ether fractions were white resins in the contrast.

Metabolites residing in the fragrant fractions of fermented and non fermented wood of the plants studied were analyzed using TLC with the aid of diagnostic reagents. They were found to be flavonoid, phenolic acids Natural product reagent(NPR), terpenoids(van H₂SO₄), catechins(van HCL) and lignans(van H₃PO₄).

Polyphenolics and terpenoids were expected to be responsible for the fragrances in the petroleum ether and ethyl acetate fractions. Removal of polyphenols as tannins with the aids of 2% NaCl reduced the fragrance in ethyl acetate fractions proving the fact of their contribution to fragrance by using (TLC).

GC/MS of petroleum ether fractions of *T. laxiflora*, *A. seyal* and *C. hartmannianum* revealed that they are mainly composed of phenolics (fragrance aromatics compounds) as major components Lup-20(29)-en-3-ol, acetate,(3. beta)(15.71%) and tetracosamethyl-cyclododecasiloxane(3.02%), and the main terpenoids compounds of petroleum ether fractions of *T. laxiflora* were Eicosamethylcyclododecasiloxane(2.69%). The phenolics of the petroleum ether fractions of *A. seyal* were petadecanoic acid(5.64%), and tetracosamethyl-cyclododecasiloxane(4.17%), and the main terponoids compounds of the petroleum ether fraction of *A. seyal* was octadecanoic acid(2.52%). The phenolic of the petroleum ether fractions of *C. hartmannianum* was 2-tert-Butyl-5-(hydroxtmethyl)-4-formylfuran(7.73%), and the main terponoids compounds was Octadecanoic acid(2.36%).

مستخلص البحث

اشجار الطلح من عائلة (Fabaceae) واشجار الصباغ والهبيل من عائلة (Combretaceae) معروفة في قارتى افريقيا وآسيا باستخداماتها الطبية العديدة. اجريت دراسة ايتنوبولوجية تعريفية لشريحة مجتمعية من النساء (100 امرأة) باستخدام استبيان لقياس معرفة الشريحة المجتمعية للاشجار الثلاث بولاية الخرطوم- السودان، مع التركيز على محليات الخرطوم والخرطوم بحري وأم درمان ونتائج التحليل الاحصائي اوضحت ان الاسم الشائع لل *A. sayal* هو الطلح بنسبة 81% وماكنتوش بنسبة 19% وان الاسم الشائع لل *C. hartmannianum* هو الهبيل بنسبة 100% وان ال *T. laxiflora* يعرف بالصباغ بنسبة 80% وبالدروت بنسبة 12% وبالكوليت بنسبة 8% اما الاسم الشائع لخشب النباتات الثلاث المتخمر بالفطريات فى المحليات الثلاث هو نخره 80% ونخاره 10% وقعور 10% علي التوالي. كما اوضحت نتائج الدراسة ان 73% من مستخدمات هذه النباتات هن متزوجات وان 27% منهن غير متزوجات كما انه لا يوجد اثر لعوامل التعليم والعمر والوظيفة اذ ان النساء المبحوثات كانت اعمارهن بين 20 و80 سنة وانهن بمختلف تعليمهن ووظائفهن يستخدمن نخرة النباتات الثلاث استخداما تقليديا للتجميل (دخان) واستخدامات اخرى. من التحليل الاحصائي اتضح ان 68% من المبحوثات يستخدمن خشب الطلح للدخان يليه الصباغ بنسبة 25% ثم الهبيل بنسبة 20% وان 50% من المبحوثات يستخدمن نخرة الصباغ كبخور يليه الطلح بنسبة 22% ثم الهبيل بنسبة 1% وايضا تستخدم نخرة هذه النباتات كطارد للبعوض وكوقود وكعلف واغراض اخرى.

تم استخلاص خشب النباتات (الطلح ، الهبيل والصباغ) المتخمرة بالفطريات بالمثانول وكانت النسبة المئوية للكيمات المستخلصة (2.99، 3.10، 3.64) وغير المتخمرة (0.64، 0.92، 2.27) على التوالي. جميع المستخلصات الميثانولية الناتجة كانت في صورة بكرة ورائحتها عطرية ولونها بني فاتح او غامض. كما استخدم كل من الايثر البترولي، واستات الايثايل، الكلوروفورم والماء لتجزئة المستخلصات الميثانولية لاختاب الطلح ، الهبيل والصباغ المتخمرة بالفطريات وغير المتخمرة ، ووجد ان النسب المئوية للمستخلص الصباغ (0.39، 1.68، 1.7) (0.05، 1.31، 0.71) ومستخلص الهبيل (0.36، 0.35، 0.49، 1.27) (0.21، 0.27، 0.09، 0.35) ومستخلص الطلح (0.89، 0.97، 0.92، 0.21) (0.05، 0.17، 0.36) على التوالي.

تم اجراء تقييم حسي باستخدام خاصية الشم للمجموعة من المبحوثين للمستخلصات المختلفة لنخرة خشب الطلح والهبيل والصباغ المتخمرة لمسح حسى للرائحة ووجد ان اقوى رائحة عطرية وجدت

فى مستخلصات الايثر البترولي واستات الايثايل وان رائحة خشب نبات الطلح اقوى بنسبة 53% ويليها الصباغ 47% اما الهبيل ليس له رائحة عطرية حيث يستخدم للمشاكل الصحية 89%.

استخدم التحليل الاحصائى بواسطة جدول تحليل المتغيرات ANOVA لدرسة مقدرة المذيبات العضوية (الايثر البترولي واستات الايثايل ، الكلوروفوم والماء) لاستخلاص المركبات الموجودة فى الخشب المتخمّر وغير المتخمّر للنباتات الطلح والهبيل والصباغ ووجد ان وزن مستخلصات الخشب المتخمّر اكبر من مستخلصات الخشب غير المتخمّر فعند مقارنة درجة الحرية المحسوبة مع درجة الحرية الجدولية وجد ان درجة الحرية المحسوبة اعلى بكثير من درجة الحرية الجدولية ولذلك فان التخمّر ونوع المذيب كان لها تأثير واضح على وزن المستخلص.

الحد الادنى للتركيز مستخلص استات الايثايل للخشب المتخمّر للهبيل المثيبط للبكتيريا (*S. aureus*, *S. typhi* and *E.coli*) كان (0.04 ، 0.04 ، 0.07) وللصباغ (0.005، 1.25، 0.04) وللطلح (1.5، 0.04، 0.005) مليجرام لكل ملتر على التوالى.

الحد الادنى للتركيز مستخلص الكلورفورم للهبيل المثيبط *C. albicans* كان 0.07 مليجرام لكل مل وللصباغ وللطلح 0.15 مليجرام لكل مل.

الحد الادنى للتركيز مستخلص الكلورفورم للهبيل المثيبط للفطر *A. niger* و *A. flavus* كان (0.3 ، 0.04) وللصباغ 0.6 مليجرام لكل مل للفطر *A. flavus* و *A. flavus* وللطلح 0.6 مليجرام لكل مل للفطر *A. niger* و *A. flavus* .

مستخلص استات الايثايل هو الاكثر نشاط كمضادات للاكسدة للنباتات الثلاثة وباستخدام طريقة DPPH وجدت النسبة المئوية للطلح (0.02±91) والصباغ (0.01±90) والهبيل (0.01±89) و IC₅₀ لمستخلص الايسايل اسيتيب للطلح (0.460±0.026) والصباغ (0.347±0.026) والهبيل (0.482±0.073) مليجرام لكل مل. مستخلص استات الايثايل هو الاكثر نشاط كمضادات للاكسدة للنباتات الثلاثة لذلك هى الاكثر احتواء على المركبات الفينولية حيث يحتوي الطلح على 424.65 مليجرام حمض الجاليك لكل جرام والصباغ 594.60 مليجرام حمض الجاليك لكل جرام والهبيل 404.96 مليجرام حمض الجاليك لكل جرام.

ثبت ان كل مستخلصات الطلح والصباغ والهبيل المتخمّره غير سامة ضد *A. salina* ماعدا مستخلص استات الايثايل والكلورفورم للطلح ومستخلص الكلورفورم للهبيل لهم سمية خفيفة.

من التحليل الفيتوكيميائي اتضح ان مستخلصات الطلح والصباغ والهبيل المتخمرة تحتوي على الالكيدات والفلويدات والتانينات والمركبات المتصبنة والجلايكوسيدات وايضا وجد ان كل مستخلصات النباتات تحتوى على التربينويدات والاسترويدات ماعدا مستخلص الكلوروفورم للصباغ ومستخلصات الكلوروفورم واسينات الايثايل الايثر البيترولولي للهبيل.

من خلال الاستبيان وباستخدام كروموتغرافيا الطبقة الرقيقة (TLC) ثبت ان العطر فى مستخلصات الطلح والصباغ والهبيل المتخمرة اقوى من غير المتخمرة وان مصدر العطر فى النباتات الثلاثة متركز فى مستخلصات البيتروليوم ايثر واستات الايثايل وهى عطور قوية وثابتة وثبت ان عطر مستخلص البيتروليوم ايثر مختلف عن عطر مستخلص استات الايثايل بالاضافة لتمييز مستخلص استات الايثايل من مستخلص البيتروليوم ايثر بالقوام الناعم واللون الاصفر ذهبى، مستخلص البيتروليوم ايثر عطرى لونه ابيض.

لمعرفة المركبات الموجودة فى مستخلصات خشب الطلح والصباغ والهبيل المتخمرة وغير المتخمرة استخدم كروموتغرافيا الطبقة الرقيقة (TLC) ثم رش ب (NPR) للتعرف على الفلانويدات والاحماض الفينولية وحمض الكبريتيك فانليلين (التربينويدات) وحمض الهيدروكلورك فانليلين (الالكيدات) وحمض الفسفوريك فانليلين (اللجان).

الفينولات العديدة والتربينويدات يتوقع ان تكون هي المسئولة عن العطر الموجود فى مستخلصات استات الايثايل و البيتروليوم ايثر, ولازالة الفينولات العديدة (التانينات) من مستخلصات استات الايثايل لخشب الطلح والصباغ والهبيل المتخمرة عند استخدام محلول كلوريدات الصوديوم 2% لوحظ ان تركيز العطر يقل فى مستخلصات استات الايثايل وذلك يبرهن ان الفينولات العديدة (التانينات) مساهمة فى الرائحة العطرية فى مستخلصات استات الايثايل واثبت ذلك بكرموتغرافيا الطبقة الرقيقة (TLC) .

وللتعرف على المركبات العطرية الرئيسية الموجودة فى مستخلص البيتروليوم ايثر لنباتات الصباغ والطلح والهبيل المتخمرة استخدم جهازكروماتوغرافيا الغاز/الطيف الكتلى (GC/MS). اتضح ان المركبات العطرية الرئيسية فى مستخلص البيتروليوم ايثر تتألف من مركبات فينولية ومركبات تربينية, اهم المركبات الفينولية للصباغ Lup-20(29)-en-3-ol, acetate, (3.beta) وكانت نسبته 15.71% و Tetracosamethylcyclododecasiloxane بنسبة 3.02% واهم المركبات التربينية Eicosamethylcyclodecasiloxane بنسبة 2.69%.

واهم المركبات الفينولية فى مستخلص البيتروليوم ايثر للطلح

Petadecanoic acid (5.64%), Tetracosamethyl-cyclododecasiloxane (4.17%),

ومركبات تربينية واهمها Octadecanoic acid بنسبة (2.52%)

واهم والمركبات الفينولية فى مستخلص البتروليوم ايثر للهيل -5-tert-Butyl

(7.73%) (hydroxymethyl)-4-formylfuran ومركبات تربينية واهمها Octadecanoic acid

بنسبة (2.36%).

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Abbreviations

CHCL ₃	Chloroform
CID	Collision induced dissociation
DAD	Diode array detector
DPPH	1,1-diphenyl-2 –pierylhydrazyle
ESI	Electro spray ionization
DMSO	Dimethyl sulf oxide
GAE	Gallic Acid Equivalent
HPLC	High performance liquid chromatography
IC ₅₀	Half inhibition concentration
Marc	Residue of the extract
MIC	Minimum inhibition concentration
MS	Mass spectroscopy
MS/MS	Tandem mass spectroscopy
<i>m/z</i>	Mass to charge ratio
NP	Normal phase
NPR	Natural product reagent
PEG	Polyethylenglycol
<i>R_f</i>	Retardation factor
RP	Reverse phase
t _R	Retention time
S.D.A	Sabouroud dextrose
SPE	Solid phase extraction
SR	Spray reagents
TLC	Thin layer chromatography
HSV-1	Herpes Simplex Virus Type 1
HIV-1	human immunodeficiency virus-1

S.N	Synonyms
ATCC	American Type Culture Collection
TM	<i>A.seyal</i> Methanol
AC	<i>A.seyal</i> Chloroform
AP	<i>A.seyal</i> Petroleum ether
AE	<i>A.seyal</i> Ethyl acetate
AA	<i>A.seyal</i> Aqueous
TM	<i>T. laxiflora</i> Methanol
TC	<i>T. laxiflora</i> Chloroform
TP	<i>T. laxiflora</i> Petroleum ether
TE	<i>T. laxiflora</i> Ethyl acetate
TA	<i>T. laxiflora</i> Aqueous
CM	<i>C. hartmannianum</i> Methanol
CC	<i>C. hartmannianum</i> Chloroform
CP	<i>C. hartmannianum</i> Petroleum ether
CE	<i>C. hartmannianum</i> Ethyl acetate
CA	<i>C. hartmannianum</i> Aqueous
WHO	the World Health Organization
LC-ESI-MS/MS	liquid chromatography Electro-Spray Ionization tandem Mass Spectrometry
mRNA	Messenger Ribonucleic acid
DNA	deoxyribonucleic acid
BHA	butylated hydroxyanisole

Table of Abbreviations

M	Methanol
C	Chloroform
P	Petroleum ether
E	Ethyl acetate
A	Aqueous
D. F.	Degree of freedom
M. S	Means Square
D	<i>Dokhan</i>
U	Fuel
B	<i>Bakhour</i>
F	Fodder
MR	Mosquito repellent
f table	Degree of freedom from table
f ratio	Degree of freedom from practice