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Hyophorbe verschaffeltii DNA Profiling, Chemical Composition of the Lipophilic Fraction, Antimicrobial, Anti-Inflammatory and Cytotoxic Activities.

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ABSTRACT

To authenticate *Hyophorbe verschaffeltii* with investigation of lipoidal matters and biological activities. DNA profiling was carried out by random amplified polymorphic DNA-PCR. Petroleum ether extract was investigated for lipoidal matters using GC-MS. Anti-inflammatory activity was assayed in *vivo* by Carrageenan-induced rat hind paw edema technique, Antimicrobial screening was done by a standard agar well diffusion method and cytotoxicity assay was measured against MCF-7 cells using the MTT Cell Viability Assay. The ten primers used for RAPD-PCR analysis produced totally 73 amplified DNA fragments and primer OPA-12 was the best sequence for dominating *Hyophorbe verschaffeltii* producing the highest hits (10).The results of the lipoidal matter investigation revealed the presence of squalene (15.40%), phytol (4.10%), myristic acid (13.20%), undecanoic acid (11.87%) and pentadecanoic acid (11.24%). Aqueous methanol extract exhibited cytotoxicity activity at IC_{50}(323.6 μg/ml) against MCF-7 cells, anti-inflammatory activity and antimicrobial activity against *Bacillus subtillus*, *Escherichia Coli*, *Pseudomonas Aeruginosa* and *Candida albicans.* The palm may be a new potential source as a natural biologically active product to be applied in pharmaceutical industries. All those biological activities and lipoidal matters have been reported for the first time in *Hyophorbe verschaffeltii* leaves.

Keywords: *Hyophorbe verschaffeltii***,** Arecaceae, DNA fingerprinting, Lipoiphilic fraction, GC/MS, Antimicrobial, Anti-inflammatory, Cytotoxicity.

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INTRODUCTION

All cultures and civilizations from ancient times to the present day have depended fully or partially on herbal medicine because of their effectiveness, affordability, availability, low toxicity and acceptability [1].The palm family (Arecaceae) has a long history of providing man with useful materials for his daily life [2]. Chemically, the family has been neglected despite of its economic importance, probably because of difficulty of collecting fresh material and getting it authenticated [3]. Arecaceae or Palm family (Palmeae), in the order Arecales, it is among the famous plant families which include genera that embrace phenolicrich species, it is a monophyletic group including 183 genera and 2364 species [4,5]. *Hyophorbe verschaffelttii* H. Wendl. is a member of the palm family (Arecaceae, sub-family Arecoideae).This palm is endemic to the Mascarene Islands, which are located to the east of Madagascar in the Indian Ocean [6,7]. From literature reviews, there is no data reported about neither the phytochemical constituents nor biological activity of *Hyophorbe verschaffelttii* H. Wendl. Accordingly, the aim of this study is to authenticate *Hyophorbe verschaffelttii* H. Wendl cultivated in Egypt by carrying out PCR sequencing and to investigate its lipoidal constituents. In addition, *in vitro* investigation of antimicrobial, cytotoxic activities and *in vivo* anti-inflammatory activity of the palm leaves extract.

MATERIALS AND METHOD

General experiment

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR system 9700 (PE Applied Biosystems). UV Polaroid camera type 57 (ASA 3000) for visualization of RAPD fragments. Data analyser software (Gel-Pro Analyser V .3.1, USA). A Perkin Elmer Cetus 480 used for the amplification of DNA. Agarose gel electrophoresis tool (Biorad Wide Mini Sib Cell) for separation of RAPD fragments according to size. The GC/MS analysis was performed using aThermo Scientific,Trace GC Ultra / ISQ Single Quadrupole MS, TG-MS fuses silica capillary column (30m, 0.251mm, 0.1 mm film thickness).For GC/MS detection, an electron ionization system with ionization energy of (70 Ev) was used. Helium gas was used as the carrier gas at a constant flow rate of 1ml/min. the injector and MS transfer line temperature was set at 280 º,GLC instrument (Hewlett Packard HP 6890 series GC system) with detector FID (flame ionization detector) for saponifiable and unsaponifiable matter in GC laboratory (Central services laboratory), National Research center, Dokki, Giza. Cytotoxicity was measured against MCF-7 cells using the MTT Cell Viability Assay.Antimicrobial assay was evaluated using the standardAgar well diffusion technique. Anti-inflammatory activity was evaluated *in vivo*by Carrageenan-induced rat hind paw edema model. Smiec Vernier Calipers (China) for measuring the paw volume.

Chemicals

RNAase (BoehringerManneheim),Taq DNA polymerase (PerkinElmer/cetus, USA, advanced Biotechnologies,UK) and Human breast adenocarcinoma cell line (MCF-7) from(ATCC Manassas, USA), Roswell Park Memorial Institute medium (RPMI-1640).Fetal Bovine Serum (FBS), Acetic acid, Penicillin/Streptomycin,Trichloroacetic acid (TCA), Trypsin, Penicillin/Streptomycin and Sulphorhodamine-B (SRB) all those chemicals purchased from(Sigma Chemical Co., St. Louis,Mo, U.S.A). Nutrient agar medium (Adwic) and CzapeksDox agar medium. Voltaren as standard anti-inflammatory drug (Novartis, Egypt), Carrageenan (Sigma, USA) was used for induction of acute inflammation in rats, Ampicillin (ADWIC,Egypt) and Fluconazole (EIPICO, Egypt) as reference antibiotic and antifungal drugs, respectively.

Plant material

Fresh plant material leaves of *Hyophorbe verschaffeltt iH.* Wendl.(Arecaceae) were collected from El-Zohreya Botanical garden, Cairo,Egypt January 2012. The plant was kindly identified by Agricultural EngineerTereaseLabib,El Orman Botanical Garden. The fresh plant leaves were completely dried in shade place at room temperature and then powdered by electric mill. The dried powders were kept in a dark place until subjected to the extraction process.

Random amplified polymorphic-DNA (RAPD)-PCR

Freeze-dried leaves of *H. verscahffeltii* were ground under liquid nitrogen to fine powder (50 mg) prior to DNA isolation. DNA extraction using the Qiagen D Neasy kit (QIAGEN Santa Clara, CA),DNA was extracted using the cetyl trimethyl ammonium bromide [1% (w/v) N-cetyl-N,N, N trimethyl ammonium bromide] method[8,9].Ten oligonucleotide primers were used for RAPD analysis in this study. PCR products were visualized on UV light and photographed using a Polaroid camera. Amplified products were visually examined and the presence or absence of each size class was scored as + or -, respectively. The banding patterns generated by RAPD-PCR marker analyses were compared to determine the genetic relatedness (Figure 1)[10,11].

GC-MS analysis of the lipophilic fractions

Extraction of lipophilic fraction

A total of 150 g of powdered plant leaves *Hyophorbe verschaffeltii* were extracted with petroleum ether, The solvent was distilled off under vacuum at temperature 45º to yield 2.7 g leaves petroleum ether extract.

Saponification of the lipophilic fraction and formation of fatty acid methyl esters

2 g of the petroleum ether residue was saponified by refluxing with 30 % alcoholic potassium hydroxide for 3 hours to yield the unsaponifiable matter (USM) as well as fatty acids fraction [12,13].The fatty acid fraction was subjected to methylation by refluxing with sulphuric acid in methanol for 2 h to yield fatty acid methyl esters (FAME)[14].The produced USM and FAME are kept in a desiccators for GC-MS analysis.

GC-MS analysis for unsaponifiable matter

Unsaponifiable matter sample (USM)was injected into gas chromatographic apparatus and separation was carried outon DB-5 fused silica (5% phenyl methyl polysiloxane). The carrier gas (helium) pressure was maintained at 13 psi and the column flow rate was 1 ml/min. The oven temperature was maintained at 50ºisothermal for 3 min, and then heated to 300º at a rate of 5º/min. The temperature of the injector during the injection was 220º.

Biological Assay

Preparation of plant extract

The air dried powdered leaves of *Hyophorbe verschaffeltii* (3 kg) were extracted by maceration with 70% methanol with occasional stirring at room temperature for 3 days. The process was repeated two times till exhaustion. The combined methanol extracts were concentrated under reduced pressure at 40º to yield (420g) dried aqueous methanol extract.

Antimicrobial Activity

Test microorganisms

Gram Positive bacteria (*Bacillus subtillus*NCIB-3610and *Staphylococcus aureus*NCTC-7447),gram negative bacteria (*Escherichia Coli* NCTC-10416and *Pseudomonas Aeruginosa*NCIB-9016) and fungi (*Candida albicans*ATCC-10231 and *Aspergillus niger*ferm-BAM C-21).

Preparation of inoculum

The isolates were inoculated on nutrient agar for bacteria and Czapeks Dox agar medium for fungi as recommended by National Committee for clinical laboratory standard NCCLS.

Evaluation of Antimicrobial Activity

200 mg of total methanolic extract of *Hyophorb verschaffeltii* was reconstituted in the least amount of DMF (Dimethyl formamide) and the volume was completed to 2 ml with DMF (100mg/ml).DMF served as a negative control. 100 mg/ml solution in dimethyl formamide (DMF) of 70% methanolic extract of *H. verschaffeltii* leaves was evaluated for its antimicrobial activity using the standard agar well diffusion technique [15,16]. Subsequently, 1 cm diameter wells were bored in the agar using the sterile cork borer and 100 *µ*l of the plant extract was pipette into the wells to allow diffusion of the extracts to the agar, they were incubated at 30º for 24 hours and 48 hours of incubation at 28ºfor fungi. Ampicillin (5mg/ml) was used as a positive control for bacteria and Fluconazole (5mg/ml) as a positive control for fungi. After incubation, Plates were examined for the presence or absence of zone of inhibition and the growth inhibition zone diameter (IZD) was measured to the nearest mm. The antimicrobial activity assay was performed as described by National Committee for clinical laboratory standard [17, 18].

Cytotoxic activity

Cytotoxicity was measured against MCF-7 cells using the MTT Cell Viability Assay and doxorubicin as a positive control.MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form a dark blue insoluble formazan crystals which is largely impermeable to cell membranes, resulting in its accumulation within healthy cells. Solubilization of the cells results in the liberation of crystals, which are then solubilized. The number of viable cells is directly proportional to the level of soluble formazan dark blue color. The extent of the reduction of MTT was quantified by measuring the absorbance at 570 nm [19]. Repeats were performed for each concentration and the average was calculated. The cell viability was expressed as percentage of control and the concentration that induces 50% of maximum inhibition of cell proliferation (IC_{50}) were determined [20, 21].

Anti-inflammatory activity

Animals

24 Adult female albino rats (each 110-160 g) were used. The animals were bred and housed under standardized environmental conditions on the pre-clinical animal house, Pharmacology Department, Faculty of Pharmacy, Azhar University. The animals were housed in-group of six per cage with free access to food and water *ad libitum* throughout the experiments. The animals were acclimatized to laboratory conditions for 10 days before beginning the experiments.

Evaluation of anti-inflammatory activity

Carrageenan-induced rat hind paw edema model described [22] was performed. This method of testing anti-inflamamtory activity depends on measuring the inhibition of edema produced acutely by injection of an irritant (phlogistic agent) into the tissues of the plantar surface of the hind paw of the rat. The experiment was done using one dose level of the aqueous methanolic extract of *Hyophorbe verschaffeltii*. Rats were fasted for 16 h before starting the experiment and divided into four groups (6 rats/ group).The pedal inflammation was induced in rat paws by injection of 0.1 ml, 1% Carrageenan suspension in 0.9% NaCl solution into the sub-plantar tissue of the right hand paw. At the beginning of the experiment, the paws thickness was measured in mm using varinier caliber. The first group was kept as a vehicle control, injected by 0.9% NaCl solution into the sub-plantar tissue of the right hand paw. While the second group injected by 0.1 ml, 1% Carrageenan suspension in 0.9 % NaCl solution into the sub-plantar tissue of the right hand paw. The third group injected intraperitionally by Voltaren (Diclofenac Na) (100mg/kg) and kept as a positive control. The fourth group was given orally (500mg/kg) of the aqueous methanolic extract of *Hyophorbe verschaffeltii*. The dose was selected based on pilot experiment in the laboratory. After 1 hour from administration, the inflammation was induced by injection of 0.1 ml, 1% w/v Carrageenan suspension in 0.9% NaCl solution [(23)]into the sub-plantar tissue of the right hand paw while the left one kept as a reference.

Paw thickness was measured using vernier caliper immediately before theinjection of carrageenan and after 1, 2, 4, 6, 8, 12 and 24 hours of carrageenan injection.

The anti-inflammatory efficacy was estimated by comparing the magnitude of paw swelling in the pretreated animals with those induced by Carrageenan only.

Statistical analysis:

Values were expressed as mean ±SEM (n=6).Statistical significance was determined using one –way analysis of variance(ANOVA) followed by Dunnet's t-test .Values of $p<0.05$ were considered significant.

RESULTS

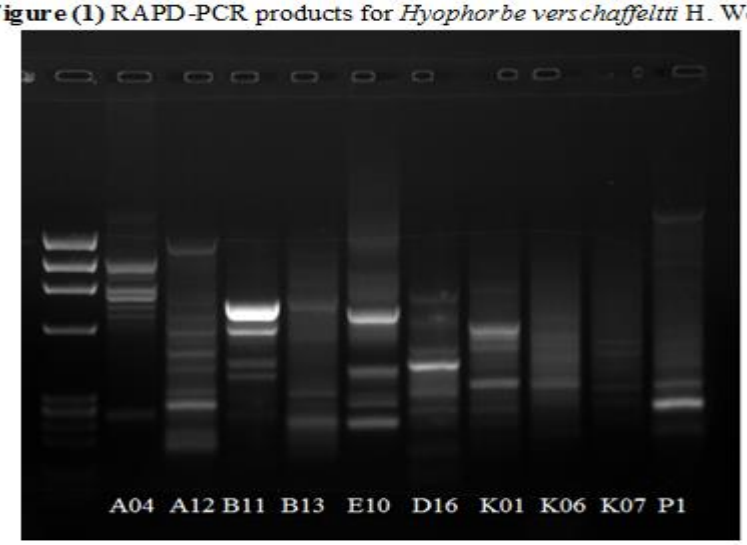
DNA profiling analysis

Total genomic DNA profiling of *Hyophorbe verschaffelttii* H. Wendl. cultivated in Egypt was performed using 10 random primers. The number of banding patterns generated by each primer was recorded to obtain the plant DNA profile, in order to compare it with previously reported phenotypic characters as well as for chemical investigations. The ten primers (*OPA-04, OPA-12, OPB-11, OPB-13, OPE-10, OPD-16, OPK-01, OPK-06, OPK-07* and *OPP-01*) of arbitrary sequences generated 73 fragments in *H. verschaffelttii* (Table 1).The obtained RAPD-PCR products are represented in Figure 1.

Table 1: Total numbers of RAPD-PCR fragments in *H. verschaffelttii*

Primer code	Sequence (5'-3')	RAPD Fragments
OPA-04	5'-AATCGGGCTG-3'	9
OPA-12	5'-TCGGCGATAG-3'	10
OPB-11	5'-GTAGACCCGT-3'	6
OPB-13	5'-TTCCCCCGCT-3'	6
OPE-10	5'-CACCAGGTGA-3'	8
OPD-16	5'-AGGGCGTAAG-3'	8
OPK-01	5'-CATTCGAGCC-3'	8
OPK-06	5'-CACCTTTCCC-3'	7
OPK-07	5'-AGCGAGCAAG-3'	4
OPP-01	5'-GTAGCACTCC-3'	7
Total		73

Figure (1) RAPD-PCR products for *Hyophorbe verschaffeltti* H. Wendl.



Lipophilic constituents of petroleum ether extract of *H. verschaffelttii* leaves

The GC-MS analysis of *H. verschaffelttii leaves* unsaponified (USM) and saponified (FAME) petroleum ether fractions revealed the presence of 21 and 32 phytochemicalcompounds, respectively, that could

contribute to the medicinal quality of the plant. The identification of the compounds was confirmed on the basis of peak area, retention time and molecular formula. The active principles with their relative retention time, molecular weight and peak area in percentage are presented in tables 2 and 3.The yield of lipoidal matter in *Hyophorbe verschaffeltii* was nearly 2.7% and the percentage of unsaponifaible matter and total fatty acids fraction was 17% and 8.5%, respectively. The most abundant component in USM is Squalene (15.40 %) $C_{30}H_{50}$ and the most abundant fatty alcohol in USM is Phytol (4.10%) $C_{20}H_{40}O$. Whereas, The Most abundant component in the saponifiable fraction of lipophilic extract of *Hyophorbe verschaffeltii* leaves is Isopropyl mysistate followed by Undecanoic acid and Pentadecanoic acid, 14-methyl, methyl ester with the concentration of 13.20%, 11.87% and 11.24%, respectively.

Table 2: GC-MS analysis of the unsaponified compounds of petroleum ether extract of *Hyophorbe verschaffeltii* leaves

Name	Relative retention time*	Molecular weight	Area %	Molecular weight
9,11-Dodecadien-1-yl acetate	0.23	224	1.52	$C_{14}H_{24}O_2$
17-(Acetyloxy)-4,4-dimethyl-7-oxoandrost-5-en-3-ylacetate	0.25	416	2.33	$C_{25}H_{36}O_5$
Junipene(Longifolene)	0.37	204	2.52	$C_{15}H_{24}$
Aristolone	0.41	218	0.92	$C_{15}H_{22}O$
Pentalene,octahydro-1-(2-octyldecyl)	0.60	362	8.23	$C_{26}H_{50}$
2H-Benzocyclohepten-2-one, Decahydro-9α-methyl-,trans	0.61	180	3.06	$C_{12}H_{20}O$
Phytol	0.62	296	4.10	$C_{20}H_{40}O$
Lanosta-7,9(11)-dien-18-oic acid, 22,25-epoxy-3,17,20-trihydroxy,ç-lactone	0.65	484	1.79	$C_{30}H_{44}O_5$
1,1,6,6-Tetracyclohexylhexane	0.95	414	1.53	$C_{30}H_{54}$
7,8-Epoxylanostan-11-ol,3-acetoxy	0.98	502	1.32	$C_{32}H_{54}O_4$
Squalene	1	410	15.40	$C_{30}H_{50}$
Dimethoxyglyceroldocosyl ether	1.2	460	0.93	$C_{27}H_{56}O_5$
3,3-ethylenedioxy-5α-Cholestane	1.24	430	1.97	$C_{29}H_{50}O_2$
Stearic acid, 3-(octadecyloxy)propyl ester	1.25	594	0.91	$C_{39}H_{78}O_3$
Methyl arjunolate	1.31	502	1.03	$C_{31}H_{50}O_5$
9-Desoxo-9-xi-hydroxy-3,7,8,9,12 pentaacetate ingol	1.32	578	0.90	$C_{30}H_{42}O_{11}$
1,4-bis(Cyclopenta-1,4-dienyl)benzene	1.33	206	1.00	$C_{16}H_{14}$
Dimethyl 4α-formyl-2,7-dihydroxy-1-methyl-8-methylenegibbane-1,10-dicarboxylate	1.35	406	1.17	$C_{22}H_{30}O_7$
9,12-Octadecadienoicacid (Z,Z)	1.38	280	1.03	$C_{18}H_{32}O_2$
3,19:7,8-Diepoxyandrostane, 17-acetoxy-3-methoxy-4,4,7-trimethyl	1.39	418	1.06	$C_{25}H_{38}O_5$
Astaxanthin	1.43	596	1.02	$C_{40}H_{52}O_4$

Relative retention time*: Relative to Squalene = 45.03 min.

Table 3: GC-MS analysis of the saponified compounds of petroleum ether extract of *Hyophorbe verschaffeltii* leaves

Name	Relative retention time*	Molecular weight	Area %	Molecular weight
7,9-di-tert-butyl-1-oxaspiro[4.5] deca-6,9-diene-2,8-dione	0.49	276	1.32	$C_{17}H_{24}O_3$
Methyl myristate	0.82	242	4.93	$C_{15}H_{30}O_2$
Methyl-11-bromoundecanoate	0.83	278	11.87	$C_{12}H_{23}BrO_2$
Methyl5,11,14-eicosatrienoate	0.91	320	0.26	$C_{21}H_{36}O_2$
9-Hexadecenoicacid, eicosyl ester, (Z)	0.94	534	2.10	$C_{36}H_{70}O_2$
Isopropyl Myristate	1	270	13.20	$C_{17}H_{34}O_2$
28-hydroxy friedelan-3-one	1.07	442	0.44	$C_{30}H_{50}O_2$
(22S)6à,11á,21-Trihydroxy-16à,17à propylmethylenedioxypregna-1,4-diene-3,20-dione	1.08	446	0.36	$C_{25}H_{34}O_7$
2-Octadecenal	1.14	266	0.41	$C_{18}H_{34}O$
Pentadecanoic acid,14-methyl,methylEster	1.17	270	11.24	$C_{17}H_{34}O_2$

Palmitic acid, ethyl ester	1.3	284	1.71	$C_{18}H_{36}O_2$
Stearic acid	1.35	284	1.06	$C_{18}H_{36}O_2$
6-tert-Butyl-o-cresol	1.36	164	4.47	$C_{11}H_{16}O$
6,9-Octadecadienoic acid, methyl ester	1.46	294	6.04	$C_{19}H_{34}O_2$
Linolenic acid, methyl ester	1.47	292	8.40	$C_{19}H_{32}O_2$
Methyl stearate	1.51	298	3.16	$C_{19}H_{38}O_2$
Decanoic acid	1.55	826	0.33	$C_{50}H_{82}O_9$
Ethyl linoleate	1.57	308	0.46	$C_{20}H_{36}O_2$
Glyceryllinolenate	1.58	352	0.74	$C_{21}H_{36}O_4$
Ethyl stearate	1.62	312	0.80	$C_{20}H_{40}O_2$
Phytofluene	1.67	542	0.19	$C_{40}H_{62}$
Methyl isoheptadecanoate	1.83	284	0.56	$C_{18}H_{36}O_2$
Octadecanoic acid, 5-hydroxy,ë-lactone	1.87	282	1.01	$C_{18}H_{34}O_2$
Trans-2- pentadecyl-4-acetoxymethyl-1,3-dioxolane	1.9	356	0.23	$C_{21}H_{40}O_4$
Adipic acid, dioctyl ester	1.94	370	0.88	$C_{22}H_{42}O_4$
9-*n*-Hexylheptadecane	2.08	324	0.26	$C_{23}H_{48}$
Methyl (Z)-13-docosenoate	2.09	352	0.26	$C_{23}H_{44}O_2$
Docosanoicacid,methyl ester	2.13	354	0.83	$C_{23}H_{46}O_2$
Tricosanoic acid,methyl ester	2.27	368	0.22	$C_{24}H_{48}O_2$
2-Octadecoxyethanol	2.36	314	0.24	$C_{20}H_{42}O_2$
Heneicosanoic acid,18-propyl,Methyl ester	2.40	382	0.50	$C_{25}H_{50}O_2$
5á-Bufa-20,22-dienolide,-14,15á-epoxy-3á,16á-dihydroxy,3-acetate	2.47	442	0.23	$C_{26}H_{34}O_6$
8,14-Seco-3,19-epoxyandrostane-8,14-dione,17acetoxy-3á-methoxy-4,4-dimethyl	2.49	420	0.3	$C_{24}H_{36}O_6$

Relative retention time*: Relative to isopropyl myristate = 13.49 min

Biological assay

Antimicrobial Activity

Antimicrobial activity of the 70% methanolic extract of *H. verschaffeltii* against the tested organisms was evaluated by measuring the zone of inhibition against the tested microbial strains, the results obtained in mm compared with the standards ampicillin and fluconazole (50mg/ml). The results are shown in Table (4**,** 5) and Figure (2)

Table 4: Inhibition zones diameter of the 70% methanolic extract of leaves of *Hyophorbe verschaffeltii*

Name of pathogen	70% methanolic extract 100mg/ml	Ampicillin5mg/ml
Bacillus subtillus	16	23
Staphylococcus aureus	no effects	20
Escherichia Coli	16	32.5
Pseudomonas aeruginosa	13.5	no effects

Table 5: Inhibition zones diameter of the total 70% methanolic extract of leaves of *Hyophorbe verschaffeltii* against the tested fungi

Name of pathohen	70% methanolic extract 100mg/ml	Fluconazole5mg/ml
Candida albicans	22	40
Aspergillus niger	no effects	no effects

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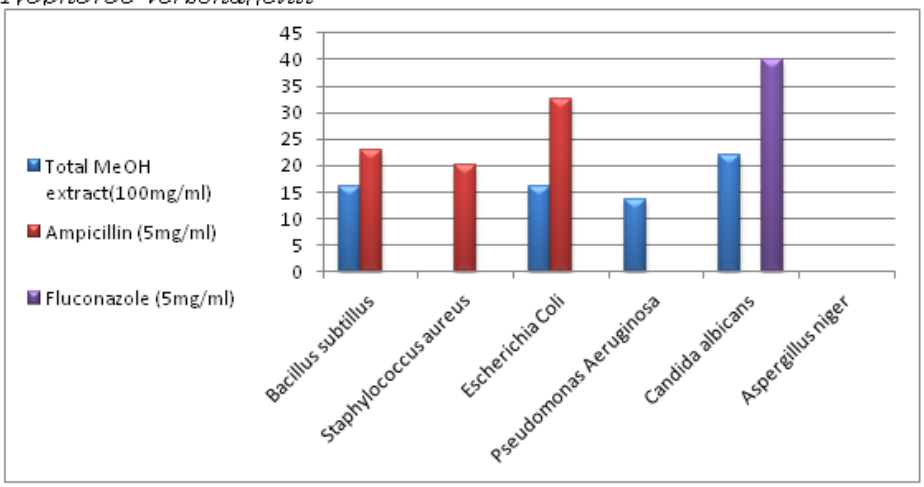
Figure (2): Antimicrobial activity of 70% methanolic extract of leaves of *Hyophorbe verschaffeltii*



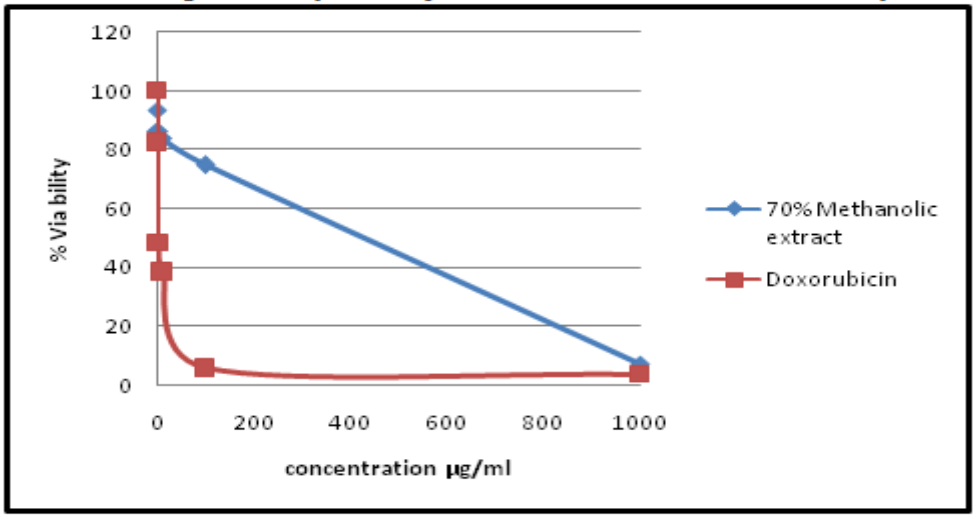
Cytotoxic activity

The Cytotoxic assay was applied with a broad range of concentrations of the 70% aqueous methanol extract of *H. verschaffeltii* leaves (from 0.-1000 µg/mL) against MCF-7 cells. The assay revealed that the extract has a weak cytotoxic activity against MCF-7 cells with IC_{50} equals 323.6 *µ*g/ml. Table (6) and Figure (3).

Table 6: Cytotoxic Activity of different concentrations of the 70% methanol extract of leaves of *Hyophorbe verschaffeltii* against MCF-7 cells using MTT assay, data expressed as the mean value of cell viability

Extract Concentration (*µ*g/ml)	Viability (% of control)	Doxorubicin (*µ*mole/ml)	Viability (% of control)
0	100	0	100
0.1	93.58	0.01	82.42
1	86.01	0.1	48.32
10	84.12	1	38.35
100	74.84	10	5.78
1000	**7.33**	100	3.54

Figure (3): Cytotoxic Activity of different concentrations of the 70% methanol extract of leaves of *Hyophorbe verschaffeltii* against MCF-7 cells using MTT assay, data expressed as the mean value of cell viability.



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Anti-inflammatory activity

The sub-planter injection of 0.1 ml, 1% Carrageenan suspension into the rat hind paw elicited an inflammation (Swelling and erythema) and a time-dependat increase in paw edema by 112.8%, 233.66% & 250% at 1st, 2nd, and 4th hours respectively, & the paw thickness was maximal by 266.83% at 6hr post carrageenan injection as compared with pre-carrageenan control values. Oral administration of methanol extract of *Hyophorbe verschaffeltii* (500mg/kg) possesses anti-inflammatory activity in carrageenan-induced rat hind paw edema model. It showed inhibition of edema formation from the 1st hour and become highly significant by 48.54% after 8 hours as compared with carrageenan control group at the same time post carrageenan injection. Voltaren (100mg/kg) showed significant inhibition of edema formation by 50.48%, 52.2% and 42.71% at 2nd, 4th and 6th hours, respectively as compared with carrageenan control group at the same time post carrageenan injection. Results are shown in Table (7) and Figure (4).

Table 7: Time course of the effect of oral administration of Voltaren, methanol extract of *Hyyophorbe verschaffeltii* leaves (500 mg/Kg), on rat right paw edema formation induced by sub-plantar injection of 1% carrageenan.

Drug	0h (basal)	1h edema (cm)(% increase)	%inhibition	2h edema (cm)(% increase)	%inhibition	4h edema (cm)(% increase)	%inhibition	6h edema (cm)(% increase)	%inhibition	8h edema (cm)(% increase)	%inhibition	12h edema(cm)(%increase)	%inhibition	24h edema (cm)(% increase)	%inhibition
Saline	0.238±0.022	0.373±0.03 (65.7%)	---	0.351±0.02 (47.47%)	---	0.367±0.07(54.2%)	---	0.238±0.02 (0%)	---	0.243±0.00 (2.1%)	---	0.193±0.016 (-18.9%)	---	0.218±0.014(-8.4%)	---
Carrageenan	0.202±0.016	0.43±0.055 (112.8%)	---	0.674±0.055• (233.66%)	---	0.707±0.040•(250%)	---	0.741±0.037• (266.83%)	-----	0.589±0.048• (191.58%)	---	0.490±0.062•(142.57%)	---	0.395±0.027•(95.5%)	---
Voltareen 100mg/kg	0.210±0.025	0.426±0.033 (102.85%)	8.82%	0.453±0.043* (115.7%)	50.48%	0.461±0.047* (119.5)	52.2%	0.531±0.064*• (152.85%)	42.71%	0.478 ±0.042• (127.61%)	33.39%	0.434 ±0.047•(106.6 6%)	25.18%	0.315 ±0.028•(50%)	47.64%
H.Verschaffeltii 500mg/kg	0.210±0.020	0.440±0.043 (109.52%)	2.91%	0.643 ± 0.13•(204.76 %)	13.36%	0.573±0.029• (172.85%)	30.86%	0.580 ± 0.056• (176.19%)	33.97%	0.417 ± 0.024*• (98.57%)	48.54%	0.377 ± 0.011•(79.52 %)	44.22%	0.345 ± 0.022•(64.28 %)	32.69%

Data represent the mean thickness of paw edema (cm) ± SEM (n=6) and % edema increase compared with the basal (zero time) values for each group in bracket.

Data were analyzed using one way ANOVA and Dunnett multiple comparison test * P<0.05 Vs Carrageenan control value at respective time point.• P < 0.05 Vs saline control value at respective time point,Percentedema inhibition was calculated compared to carrageenan control group.

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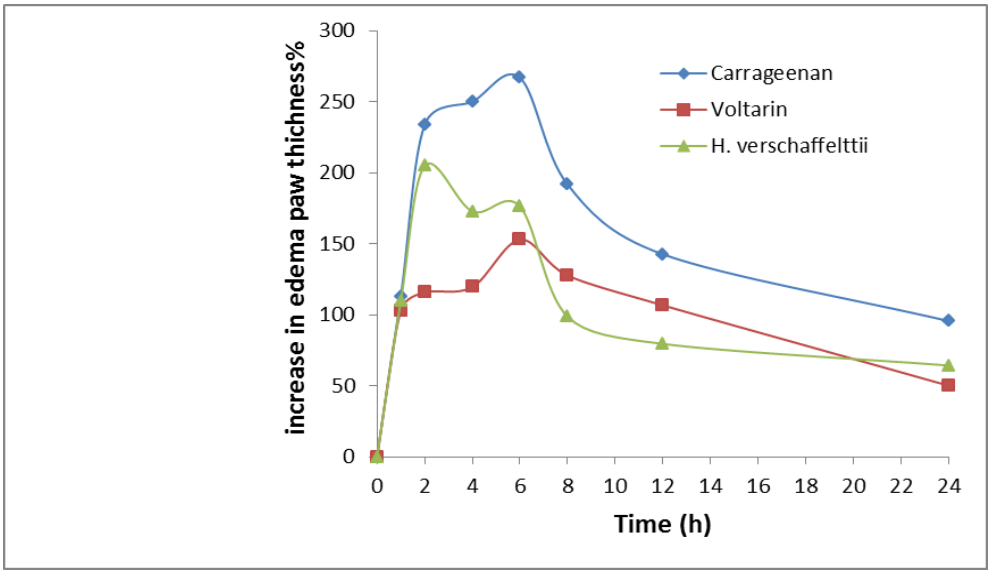

Figure 4: Time course of the effect of oral administration of Voltaren and methanol extract of *Hyyophorbe verschaffeltii* leaves(500 mg/Kg), on rat increase in edema paw thickness induced by sub-plantar injection of 1% carrageenan

DISCUSSION

The DNA profiling analysis of *Hyophorbe verschaffelttii H.* Wendl., the number of RAPD-PCR fragments indicates that the ten primers were reproduced. The DNA amplified with RAPD technique using primer A-12primer is the most characteristic showing 10 fragments while primer K-07 is the least characteristic showing 4 fragments only. It is noteworthy that primer *A-04* showed good dominating for *Hyophorbe verschaffeltii* producing 9 amplified DNA fragments and primers E-10, D-16 and K-01 produced 8 amplified DNA fragments while primers K-06 and P-01 showed moderate dominating producing 7 amplified DNA fragments. Whereas Primers B-11 and B-13 produced only 6 amplified DNA fragments. Therefore, primer A-12 was the best sequence for dominating *Hyophorbe verschaffelttii* H.Wendl cultivated in Egypt. GC-MS spectral data analysis of *Hyophorbe verschaffelttii* H.Wendl leaves unsaponified (USM) and saponified (FAME) petroleum ether fractions revealed the presence of 21 and 32 phytochemicl compounds, respectively. It revealed the presence of series of hydrocarbons ranging from Junipene (C15) to tetracyclohexane (C30) and presence of series of fatty acids ranging from myristic acid (C14:0) to Tricosanoic acid (C23:0) The most abundant component in USM is Squalene (15.40 %),followed by Pentalene,octahydro-1-(2-octyldecyl)(8.23%), Phytol(4.10%).The Most abundant FAME is Isopropyl mysistate (Mysristic acid C14:0) followed by Undecanoic acid C11:0 and Pentadecanoic acid, methyl, 14-methyl ester (Pentadecanoic acid C15:0) with the concentration of 13.20%, 11.87% and 11.24%, respectively. Squalene is a polyunsaturated triterpene containing six isoprene units and is a biochemical precursor of cholesterol and other steroids. It is a highly effective singlet oxygen-scavenging agent (24).It has also been found to have protective activity against several carcinogens [25]. It is also capable of suppressing the growth of tumor cells [26]. Such protective, anticarcinogenic and differential activities in normal tissue against chemotherapeutic agents [27]. Phytol is a cyclic diterpene, a member of the group of branched-chain unsaturated alcohols; it is the product of chlorophyll metabolism in plants. It possesses antioxidant activity associated with antinociceptive activities [28]. Myristic acid and Undecanoic acid possess antioxidant and anti-inflammatory effect through inhibition of (COX-I) and (COX-II) inflammatory mediators [29] and 14-Methyl-pentadecanoic acid (11.24%) possesses antioxidant and antimicrobial effects [30].

Antimicrobial activity of *H. verschaffelttii* H. Wendl revealed that 70% methanolic extract demonstrated moderate bactericidal activity against *Bacillus subtillus*, *Escherichia Coli* and strong bactericidal activity against *Pseudomonas aeruginosa* relative to positive control(Ampicillin) and exhibited inhibition zones of 16, 16, 13.5 mm diameter, respectively, and has moderate antifungal activity against *Candida albicans* relative to positive control (Fluconazole) and exhibited inhibition zones of 22 mm diameter. In this study we report for the first time the antimicrobial activity of *Hyophorbe verschaffeltii* total methanolic extract (100mg/ml) against Gram positive bacteria (*Bacillus subtillus*), Gram negative bacteria (*Escherichia Coli* and *Pseudomonas Aeruginosa*) and fungus (*Candida albicans*).

Cytotoxic activity was observed against human breast adenocarcinoma cell line (MCF-7) for the 70% methanol extract of leaves of *H. verschaffeltii*. The highest activity was for concentration of 1000 µg/ml of 70% methanol extract of leaves of *Hyophorbe verschaffeltii* that shows viability of 7.33 %.The concentration (µg/ml) of 70% methanol extract of leaves of *H. verschaffeltii* necessary to produce 50% inhibition equals 323.6 µg/ml. Therefore, *H. verschaffeltii* extract had weak cytotoxicity activity against MCF-7 cells, IC50 = 323.6 µg/ml.

Anti-inflammatory activity by comparing the results of voltaren and *H.verschaffeltii* methanol extract, it showed that the MeOH extract has long-term anti-inflammatory activity than voltaren as it shows continuous and significant inhibition of edema by 48.54% & 44.2% at 8^{th}& 12 hours, respectively as compared with voltaren group at the same time, which makes inhibition only by 33.39% & 25.18% at 8^{th}& 12 hours.

The present work studied the biological activities and phytochemical constituents of *H. verschaffeltii* cultivatedin Egypt for the first time.

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