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RESEARCH ARTICLE

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Antimicrobial activity, cytotoxicity, and phytochemicals screenings of *Epipremnum aureum* (Linden and Andre) G. S. Bunting extracts

ABSTRACT:

The aim of this study is to determine the antimicrobial activity and cytotoxicity of aqueous, ethanolic and acetone extracts of different plant parts of *Epipremnum aureum* (leaves blades, petioles, stems, and roots). Antimicrobial activity was carried out against Gram negative bacterium (*Escherichia coli*), Gram positive bacterium (*Staphylococcus aureus*), filamentous fungus (*Aspergillus flavus*) and yeast (*Candida albicans*). *A. flavus* was resistant to all extracts. Root extracted by acetone proved to be the most effective antimicrobial extract. The Minimum Inhibitory Concentration (MIC) values of acetone root extract of *E. aureum* against *E. coli*, *S. aureus* and *C. albicans* were 3, 5, and 9 mg/ml, respectively. The *in vitro* cytotoxicity of different concentrations of *E. aureum* acetone root extract was assayed against human liver cancer cell line (HEPG-2) and found that the most effective concentration was at 50 µg/ml and the IC_{50} value was 36.7 µg/ml. Gas Chromatography Mass Spectroscopy (GC-MS) was used for phytochemical screening of acetone root extract. Twenty-one organic compounds were detected with different retention times. They were carbohydrates, fatty acids, phenols, alcohols, vitamins, alkaloids and flavonoids. Patchoulol represented the highest percentage of phytochemicals followed by myristic and palmitic acids.

KEY WORDS:

Epipremnum aureum, Antimicrobial activity, MIC, Cytotoxicity, Phytochemicals.

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INTRODUCTION:

Araceae is a big plant family consisting of around 105 genera and about 3000 monocot species (Saswati *et al*., 2013). Plants belonging to Araceae range from tiny floating aquatic plants to forest climbers. Many species are cultivated for their decorative flowers or foliage and others for their nutrition value (Meshram and Srivastava, 2015).

Epipremnum aureum (Linden and Andre) G. S. Bunting is a popular ornamental house plant belongs to Araceae. This plant is native to South eastern Asia and New Guinea. It was known for its capacity for removing indoor pollutants such as xylene, formaldehyde and benzene. It was also known, commonly, as the Golden Pothos, money plant, silver vine, etc. It is a climbing evergreen shrub which has pretty variegated foliage and aerial roots (Srivastava *et al*., 2011; Arulpriya and Lalitha, 2012; Mehta *et al*., 2013; Das *et al*., 2015; Ott and Mustapich, 2017).

Antimicrobial agents are substances that kill or prevent the microbial growth like bacteria and fungi (Choudhury and Choudhury, 2011). Previous studies revealed that drugs derived from plants are useful as antibiotics, antioxidants and anti-inflammatory agents (Mathur *et al*., 2011).

Many studies showed that many plants of Araceae had significant activities against some pathogenic bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Saswati *et al*., 2013). This may be due to the presence of particular phytochemicals such as flavonoids, alkaloids, glycosides, etc. in most members of Araceae.

E. aureum showed wide spectrum of antimicrobial activities against many pathogens. Aqueous, ethanol, methanol and acetone extracts of roots and leaves of *E. aureum* displayed antibacterial activities against gram negative (*E. coli)* and gram positive (*Micrococcus luteus*, *Bacillus subtilis* and *B. cereus)* bacteria (Srivastava *et al*., 2011).

Phytochemical analysis of methanolic extract of leaves of *E. aureum* detected the occurrence of flavonoids, alkaloids, saponins,

triterpenoids and tannins (Mehta *et al*., 2013). They have medicinal importance due to their activities as antibacterial, antifungal, calming and relaxation effect (Srivastava *et al*., 2011; Meshram and Srivastava, 2014).

The present study was planned to evaluate antimicrobial activity and cytotoxicity of different extracts of *Epipremnum aureum* plant parts. Phytochemical screening of the most active extract was also done.

MATERIAL AND METHODS:

Preparation of plant extracts:

Fresh mature plants were collected and identified by the Herbarium of Botany and Microbiology Department, Cairo University, Giza, Egypt. Plant materials (Blades, petioles, stems and roots) were firstly washed with tap water 3 times followed by distilled water and then dried at 50°C for overnight (Sen and Batra, 2012). Dried plant materials were crushed to fine powder and then were extracted with different solvents (Distilled water, ethanol and acetone) using shaker at 120 rpm for 24 hours. The extracts were filtered, concentrated and evaporated to dryness. Residues were stored for subsequent analysis.

Assay of antimicrobial activity:

Antimicrobial activities of the tested samples were determined using Kirby-Bauer disc diffusion method (Bauer *et al*., 1966). The test was done against Gram positive bacterial species (*Staphylococcus aureus*), Gram negative bacterial species (*Escherichia coli*), yeast (*Candida albicans*) and filamentous fungal species (*Aspergillus flavus*). Meuller-Hinton agar medium was used for test of bacteria and Czapek-Dox`s agar medium was used for fungi, while Sabouraud Dextrose agar media was used for yeast.

Plates inoculated with *A. flavus* were incubated at 25ºC for 48 hours; while plates inoculated with *S. aureus* and *E. coli* were incubated at 35 - 37ºC for 24 - 48 hours. *C. albicans* was incubated at 30ºC for 24-48 hours. Blank paper disks (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated with 10 µl of tested plant extract with concentration of 100 mg/ml and placed on the surface of agar media.

Standard discs of Ampicillin (Antibacterial agent) and Amphotericin B (Antifungal agent) were used as positive controls at concentration 20 mg/ml for antimicrobial activity but discs impregnated with 10 µl of solvent (DMSO) were used as negative controls. At the end of incubation period, the diameters of the inhibition zones were measured in millimetres.

Determination of Minimum Inhibitory Concentration (MIC):

MIC values of acetone root extract of *E. aureum* were determined by using agar dilution method (CLSI, 2006) against *E. coli*, *S. aureus* and *C. albicans*. Serial dilutions from the extract were prepared and mixed each with 5 ml of the bacterial suspensions or yeast suspension, then added to agar plates and incubated. The developing colonies (cfu/ml) were counted for each concentration. MIC values were determined as the lowest concentration of the extract that inhibited the visible growth of the microorganisms after incubation period.

In vitro cytotoxicity assay:

Analysis was done at National Cancer Institute, Cairo, Egypt. *In vitro* cytotoxicity of acetone root extract of *E. aureum* was performed by using Sulfo-Rhodamine-B (SRB) assay against human liver cancer (HEPG-2) cell line using different concentrations of extract (0, 12.5, 50, and 100 µg/ml). Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 hours before treatment with the extract to allow attachment of cell to the wall of plate. Different concentrations of the extract were added to the cell monolayer; triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the extract for 48 hours at 37°C under atmosphere of 5% CO_2. After 48 hours, cells were fixed, washed and stained with SRB stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Colour intensity was measured in an ELISA reader. The surviving fraction and IC_{50} values were determined (Skehan *et al*., 1990). The results were compared to standard anticancer drug (DOX).

IC_{50} is defined as the concentration which results in a 50% decrease in cell number as compared with that of the control cultures in the absence of an inhibitor (Sun *et al*., 2011).

Phytochemical screenings of acetone root extract (GC-MS analysis):

Analysis was done at Agricultural Research Centre, Giza, Egypt. The analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD Agilent 7000) equipped with an apolar Agilent HP- 5 ms (5%- phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm i.d. and 0.25 µm film thickness). The carrier gas was helium with linear velocity of 1 ml / min. The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with reported in the literature (Santanal *et al*., 2013).

Statistical analysis:

Data were analysed by one-way analysis of variance (ANOVAs) using SPSS

statistical program. The differences were compared by the Duncan's Multiple Range Test (DMRT) with the significance set at $p \leq 0.05$.

RESULTS AND DISCUSSION:

Antimicrobial activity:

Antimicrobial activities of aqueous, ethanolic and acetone extracts of different parts of *E. aureum* were determined using disc diffusion method against *E. coli*, *S. aureus*, *A. flavus* and *C. albicans*.

As shown in figure 1, *E. coli* was inhibited by all plant extracts. *S. aureus* was sensitive to all plant extracts except aqueous and ethanolic extract of leaves blades. Regarding to antifungal activities of plant extracts, *A. flavus* was resistant to all types of extracts. Meanwhile, *E. aureum* extracts exerted no activity on *C. albicans* except all root extracts and acetone extract of stems.

Concerning solvents of extraction, it was observed that acetone extracts had the highest significant antimicrobial activity, followed by ethanol extracts, while aqueous extracts had the least antimicrobial activity. This proved that ethanol and acetone extraction activated the exudation of the antimicrobial materials from all plant parts, so it caused more inhibition.

Different plant parts exhibited various antimicrobial potentialities depending on the extractors. Comparing activities of plant parts extracted with acetone, it was obvious that root extract showed significant antimicrobial activity followed by stem, then petiole and finally with leaf blade (Fig. 1). Consequently, acetone root extract was selected for the next experiments.

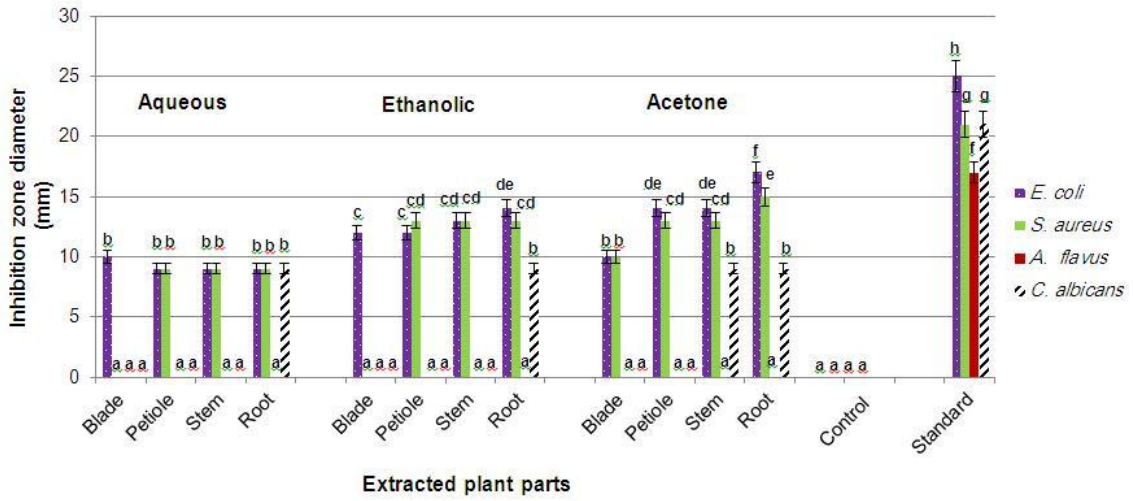


Fig. 1. Antimicrobial activities of different extracts of *E. aureum* against some microbial pathogens.

- Standard for bacteria: Ampicillin (20 mg / ml).
- Standard for fungi: Amphotericin B (20 mg / ml).
- Values are mean of triplicate readings (mean ± SD).
- Mean values with different letters are significantly different at 5% level according to Duncan's multiple range test.

In this field, Meshram and Srivastava (2015) indicated that each part of this plant possesses antibacterial, anti-termite and antioxidant properties. Mehta *et al*. (2013) reported an antimicrobial activity of methanolic extract of *E. aureum* leaves against *C. albicans*, *P. aeruginosa*, *S. aureus*, *S. mutans*, *S. typhi* and *S. paratyphi* A. Also, Sonawane *et al*. (2011) found that aqueous extract of *E. aureum* leaves exhibited significant antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans*.

MIC determination:

The MIC values of acetone root extract of *E. aureum* were detected using agar dilution method against the susceptible microorganisms *E. coli*, *S. aureus* and *C. albicans* (Table 1). It was 3 mg/ml for *E. coli*, 5 mg/ml for *S. aureus* and 9 mg/ml for *C. albicans*. Mehta *et al*. (2013) found that the MIC values of the hot methanolic extract of *E. aureum* against *P. aeruginosa, S. typhi,* and *S. paratyphi* A were in the range between 3 - 6 mg/ml. The MIC values of *E. aureum* aqueous extract were determined by Sonawane *et al*. (2011) against *E. coli*, *S. aureus* and *C. albicans* to be 25, 25, and 50 µg/ml, respectively.

Table 1. Determination of MIC values of acetone root extract of *E. aureum* against microbial species.

Microbial species	MIC value (mg/ml)
E. coli	3
S. aureus	5
C. albicans	9

***In vitro* cytotoxicity:**

The *in vitro* cytotoxicity of acetone root extract of *E. aureum* was investigated against human liver cancer cell line (HEPG-2) by using SRB assay method. Results in figure 2 referred to the cytotoxic effects of different concentrations of acetone root extract. The most efficient concentration was at 50 µg/ml. The IC_{50} value was determined at 36.7 µg/ml.

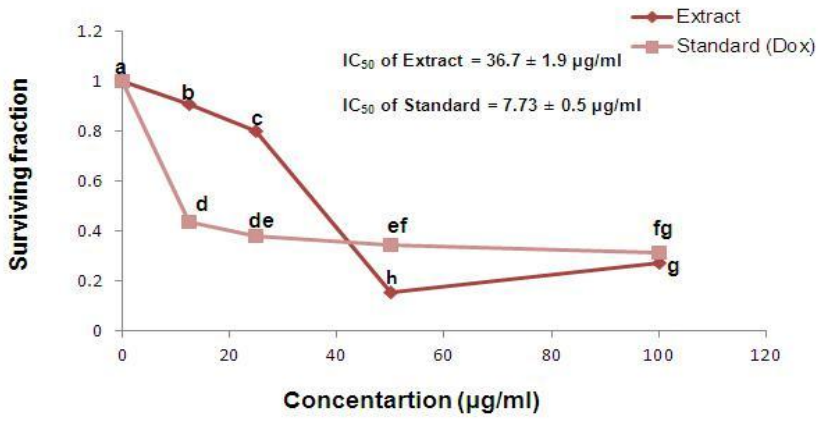


Fig. 2. *In vitro* cytotoxicity of acetone root extract of *E. aureum* compared to standard anticancer drug (DOX) against human liver cancer cell line (HEPG-2).

- Mean values with different letters are significantly different at 5% level according to Duncan's multiple range test.
- IC_{50} was expressed as mean ± SD.

In relation to our study, *Pothos scandens* L. is a medicinal plant which has been usually used for curing several diseases including cancer. Its 50% hydro-ethanolic extract showed significant cytotoxic activity against MCF-7 cell lines using LDH leakage assay. It can be considered as a potential candidate for anticancer drug research (Jethinlalkhosh *et al*., 2017).

Phytochemical analysis:

Phytochemical analysis of acetone root extract was carried out by using gas chromatography mass spectroscopy (GC-MS). As displayed in figure 3 and table 2, twenty-one organic compounds were detected in acetone root extract of *E. aureum* with different retention times. Detected compounds were carbohydrates, fatty acids, phenols, alcohols, vitamins, alkaloids and flavonoids. Meshram and Srivastava (2016) detected the presence of carbohydrates, proteins, steroids, glycosides, alkaloids, saponins, phenols, flavonoids and amino acids in methanol extracts of different explants of *E. aureum* (leaves, stems and roots). Moreover, Meshram *et al*. (2015) observed that *E. aureum* leaves extract was very rich in alkaloids and twenty-six different alkaloids were detected by using GC-MS.

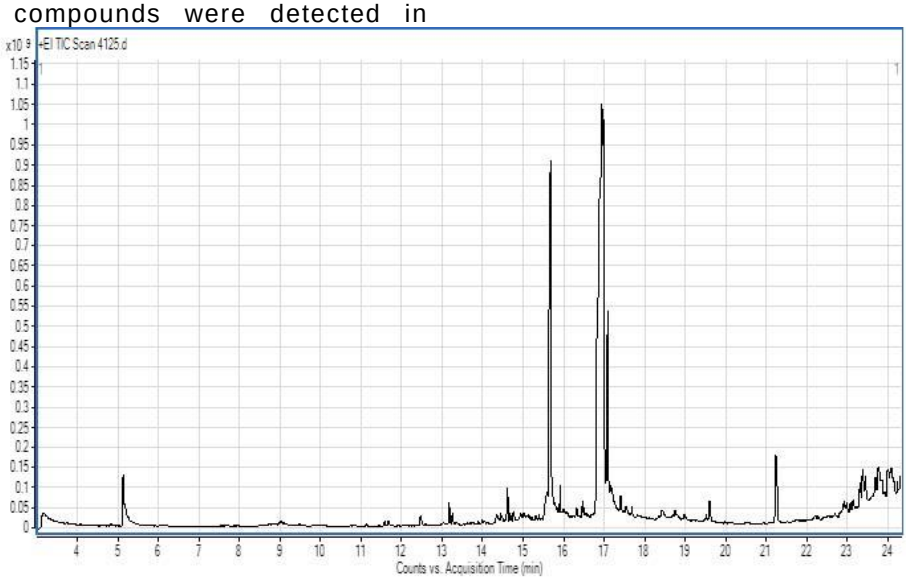
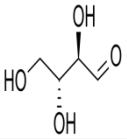
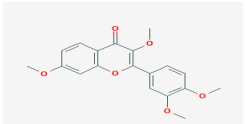
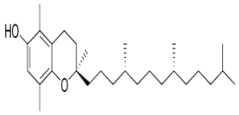
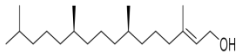
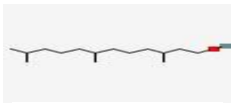
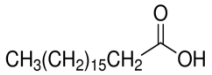
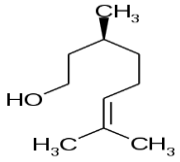
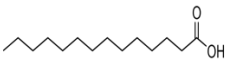
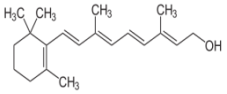
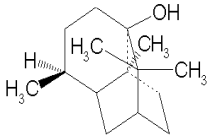
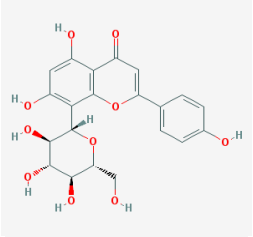
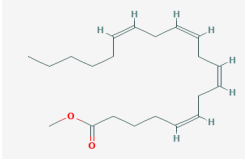
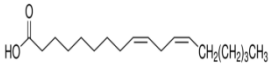
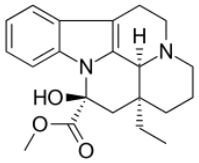
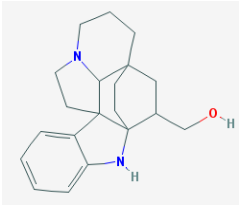
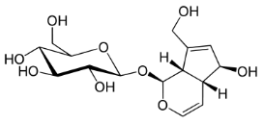
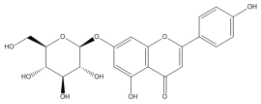
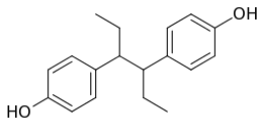


Fig. 3. GC-MS chromatogram of acetone root extract of *E. aureum.*

Table 2. Phytochemical analysis of acetone root extract of *E. aureum* using GC-MS.

No	RT (min.)	Name	Chemical Formula	Structure	Area sum %
1	5.15	D-(-)-Erythrose	$C_4H_8O_4$		3.36
2	11.1	Tetramethyl fisetin	$C_{19}H_{18}O_6$		1
3	11.56	Oleic acid	$C_{18}H_{34}O_2$		0.56
4	11.6	ß-Tocopherol	$C_{28}H_{48}O_2$		0.52
5	13.15	Phytol	$C_{20}H_{40}O$		0.62
6	13.23	Hexa-hydro-farnesol	$C_{15}H_{32}O$		0.56
7	14.33	Stearic acid	$C_{18}H_{36}O_2$		0.73
8	14.6	ß-Citronellol	$C_{10}H_{20}O$		0.69
9	15.6	Myristic acid	$C_{14}H_{28}O_2$		14.74
10	16.29	Retinol	$C_{20}H_{30}O$		0.87
11	16.45	Arachic alcohol	$C_{20}H_{42}O$		0.8
12	16.92	Patchoulol	$C_{15}H_{26}O$		50.87
13	17.06	Palmitic acid	$C_{16}H_{32}O_2$		6.11
14	17.66	Apigenin 8-C-glucoside	$C_{21}H_{20}O_{10}$		1.97
15	18.4	Arachidonic acid methyl ester	$C_{21}H_{34}O_2$		1.99
16	18.72	Linoleic acid	$C_{18}H_{32}O_2$		1.57

17	19.6	Vincamine	$C_{21}H_{26}N_2O_3$		0.72
18	21.23	Aspidofractinin-3-ylmethanol	$C_{20}H_{26}N_2O$		3.11
19	22.9	Aucubin	$C_{15}H_{22}O_9$		1.91
20	23.36	Apigenin 7-glucoside	$C_{21}H_{20}O_{10}$		3.73
21	23.76	Hexestrol	$C_{18}H_{22}O_2$		3

RT: retention time.

Many fatty acids like palmitic, linoleic, oleic, stearic and myristic acids were detected in acetone root extract of *E. aureum* (Table 2).

They were known to have potential antibacterial and antifungal properties (McGaw *et al*., 2002; Seidel and Taylor, 2004; Agoramoorthy *et al*., 2007).

Patchoulol was detected with the highest percentage followed by Myristic acid then palmitic acid in the acetone root extract of *E. aureum* (Table 2). Patchoulol is a type of sesquiterpenes which were known by their potent anticancer, antiviral and antibiotic properties, as well as their characteristic flavours and aromas (Asadollahi *et al*., 2008).

CONCLUSION:

In the present study, all *E. aureum* extracts had antimicrobial activities where acetone root extract was the most effective one. It also had cytotoxic activity. This may be due to the presence of phytochemical compounds such as patchoulol and some fatty acids.

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النشاط المضاد للميكروبات والسمية الخلوية وفحص الكيماويات النباتية لمستخلصات نبات البوتس

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هدفت الدراسة إلى تقدير النشاط المضاد للميكروبات والسمية الخلوية لمستخلصات نبات البوتس عن طريق الماء والايثانول والاسيتون من اجزاء النبات المختلفة (الاوراق، السيقان والجذور). تم قياس النشاط المضاد للميكروبات ضد البكتيريا سالبة الجرام (Escherichia coli)، البكتيريا موجبة الجرام (Staphylococcus aureus)، الفطر الخيطي (Aspergillus flavus) والخميرة (Candida albicans). كان الفطر الخيطي مقاوما لجميع المستخلصات. ثبت أن الجذر المستخلص بواسطة الأسيتون هو المستخلص الاكثر فعالية. كانت قيم التركيز الأدنى للثبيط (MIC) لمستخلص جذور الأسيتون لنبات البوتس ضد *E. coli* و *S. albicans* و *aureus* هي 3 و 5 و 9 mg / ml ، على التوالي. تم اختبار السمية الخلوية في المختبر لتركيزات مختلفة من مستخلص جذر الأسيتون لنبات البوتس ضد خلايا سرطان الكبد البشري (HEPG-2) ووجد أن التركيز الأكثر فعالية كان عند 50 ميكروغرام / مل وكانت قيمة IC_{50} هي 36.7ميكروغرام / مل. تم استخدام الطيف الكتلي للغاز (GC-MS) لفحص الكيمياويات النباتية لمستخلص جذور الأسيتون. تم اكتشاف احدى وعشرون من المركبات العضوية مع أوقات احتفاظ مختلفة. كانت هذه المركبات عبارة عن كربوهيدرات وأحماض دهنية وفينولات وكحولات وفيتامينات وقلويدات وفلافونيدات. مثل patchoulol أعلى نسبة من المواد الكيميائية النباتية تليها حمضي myristic و palmitic