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RESEARCH ARTICLE

Chemical Composition of the Essential oil of The Leaves of *Pimenta diocia* (L.) Merr. & *Pimenta racemosa* (Mill.) cultivated in Egypt and Evaluation of Their in-vitro Antioxidant and Antidiabetic Activities

Heba Abd-Elhady El-gizawy[1], Yasser Omar Mosaad[2], Naglaa Abd El Khalik Gobba[3] and Mohammed Abdalla Hussein[4*]

Abstract

The aim of the study is to identify and characterize the chemical composition of the essential oil of both leaves of *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) as well as to evaluate their *in-vitro* antioxidant and anti-diabetic potency. Both leaves essential oil was analyzed by GC-MS analysis. Different *in-vitro* antioxidant tests were employed, namely, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric thiocyanate (FTC), ferric reducing antioxidant power, thiobarbituric acid (TBA) and β-carotene-linoleate bleaching assay. Also, the present work aims to evaluate the α-amylase and α-glucosidase inhibition as well as glucose uptake by yeast cells of essential oils. Essential oil analysis of the leaves of *Pimenta racemosa* (Mill.) showed high amounts of eugenol (37.95%), β -Myrecene (21.01%), α-Pinene (17.82%), linalool (6.15%) and limonene (5.93%). GC-MS data of the leaves of *Pimenta diocia* essential oil revealed the presence of eugenol (30.17%), limonene (17.24), α-Pinene (16.78%), linalool (9.71), 1,8 cineole (8.31%) and β-myrecene (5.21%). *Pimentadiocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil was found to exert antioxidant effect using various methods. In most of the oil samples and assays the antioxidant activity was higher than the one revealed by the positive control BHT. Both plants leaves essential oil showed potent inhibition of α-Amylase at concentration 2.00 mg as it was inhibited by (75 and 63 %); with IC_{50} (0.95 and 1.13); respectively as well as inhibition of α-glucosidase enzyme by (61.42 and 53.00%) with IC_{50} (3.17 and 4.25); respectively. Also the percentage of glucose uptake by *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil at 2.00 mg/ml in the presence of 25 mM glucose is (63.49% and 49.61%); respectively. Conclusion: the present study clearly identified the Egyptian chemotype of *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil, it also displayed safe and promising antidiabetic and antioxidant properties. Therefore the essential oil of both species can be utilized as a natural antioxidant and antidiabetic as well as health benefits.

Keywords: Pimenta diocia (L.) Merr; racemosa; essential oil; antioxidants; in-vitro antidiabetic; α-amylase; α-glucosidase; glucose uptake; yeast cells

*Correspondence: Prof.husseinma@o6u.edu.eg
Full list of author information is available at the end of the article

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[4]Biochemistry Department, Faculty of Applied Medical Sciences, October 6 University, Sixth of October City, 12585, Egypt. Tel.: +2(0122) 4832580, Fax: +2 (02) 38353270

Introduction

Pimenta diocia (L.) Merr. and *Pimenta racemosa* (Mill.) belongs to family Myrtaceae ,which constitutes about 142 genra and more than 5500 species; trees and shrubs of this family with conspicuous oil glands, the genous Pimenta is composed of about 15 species, mostly found in the Caribbean region of America. Myrtaceae has an economic value because species of the family used as a source of timber, as a source of edible fruits, as spices and as a flowering ornamental [1].

Essential oils of *Pimenta diocia* (L.) Merr. have been used as antibacterial, antifungal, antioxidant and insecticidal agents [2]. Kapoor [3] has shown that about 3000 essential oils are known commercially important for the pharmaceutical, agronomic, food, sanitary products [4], cosmetic [5], perfume and make-up industries [6].

Pimenta racemosa (Mill.) has been widely used in cosmetics due to the content of volatile essences, especially in the formulations such as aftershave lotions, soaps, perfumes and hair treatments [7–10] . Regarding biological properties, the essential oil of this species has been studied for antioxidant [10, 11], insecticide [12], antibacterial [13] and antifungal [14] activities.

Nesrin et al. [15] showed that methyleugenol is the main component from *Pimenta racemosa*. As an extension of our studies on the extraction and medicinal importance evolution of the biologically active essential oil [16] , we now wish to explain the chemical analysis of Egyptian *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) essential oil by GC/MS with the aim to evaluate the influence of environmental factors on the chemical composition of the leaves of both species of the plant cultivated in Egypt. Also, the present study was extended to investigate the in vitro antioxidant and antidiabetic activity of the eseential oil of the leaves of *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.).

We choose to test for the antidiabetic activity because as we know; Diabetes mellitus is one of the most common endocrine metabolic disorders worldwide which caused significant mortality due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke, and peripheral vascular disease) complications [17] . Nowadays, some medicinal plants have been reported to be useful in diabetes and have been used empirically as antidiabetic and antihyperlipidemic remedies [18] . Plants have been always a very good source of drugs and many of the currently available drugs have been derived directly or indirectly from plant origin. The ethnobotanical information suggest that about 800 plants may possess anti-diabetic potential among all of which are Momordica charantia, Pterocarpus marsupium and Trigonella foenumgraecum have been reported to be beneficial for treatment of type 2 diabetes [19, 20] .

Full list of author information is available at the end of the article.

Material and Methods

Plant material

Leaves of *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) were collected in October 2017 from Zohria garden in Zamalek district and were identified by Dr. Ahmed Wahba, Executive manager of Zohria Garden. Voucher specimens were kept at the Herbarium of Faculty of Pharmacy, October 6 University No. (201710/A & B). The leaves were subjected to hydro distillation at atmospheric pressure, for three hours, using Clevenger- type apparatus. Both distilled oils were dried over anhydrous Na_2SO_4 and the yields (v/m) of the oil were calculated, stored at 0-10 ^oC in the dark for further analysis.

Materials and reagents

2, 2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulfate, phosphate-buffered saline (pH 7.4), acetate buffer (pH 3.6), 2,4,6-tripyridyl-*s*-triazine, hydrochloric acid, ferric chloride, linoleic acid, butylated hydroxytoluene (BHT), beta-carotene Type 1 (95%), DPPH (2,2-phenyl-1-picryl-hydroxyl) and thiobarbituric acid (TBA) were obtained from Sigma (St Louis, MO, USA), TPTZ (2, 4, 6- tripyridy-*s*-triazine) and ferric chloride from HmbG Chemicals (Germany), while ammonium thiocyate was from AJAX Chemical (Aubur, Australia), and ferrous chloride and ferrous sulphate ($FeSO_4$), Acarbose (Bicon Ltd), α-glucosidase (SRL), maltose (Loba cheme), Glucose assay reagent (Agappe Diagnostics), α-amylase (SRL) were from BDH (England).

Methods

GC/MS analysis: The analysis of both oil leaves samples was performed by gas chromatography coupled to mass selective detector using a Hewlett Packared G1800A GCD System coupled to an HP automatic injector 7673A. Column HP-5-MS (30 m x 0.25 mm i.d); carrier gas, He 1.0 ml /min.; injector temperature, 250^{o}C; detector temperature, 280^{o}C; oven temperature program: 5 min isothermal at 35^{o}C, then programmed 35-250^{o}C at 7^{o}C/min; ion source 70eV; scan mass range (30-450)*m/z*. Identification of the oil constituents was achieved by library search on a Willey 275 L GC-MS data base and by comparing their retention indices and mass fragmentation patterns to those of available references as well as published data.

Series of authentic n-alkanes was subjected to GLC analysis under the same experimental conditions and the retention indices (Kovat's indices) of the oil constituents were calculated.

Abbe's refractometer: For measuring the refractive indices of the oil samples.

Specific gravity bottle: For determining the specific gravity of oil samples.

In-vitro antioxidant activity

DPPH radical scavenging activity: Free radical scavenging activity of *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil was evaluated using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical according to the method of Shimada *et al* [21] . The percentage of free radical scavenging activity was calculated based on the following equation:

Scavenging activity (%) = 1- [A_s/A_c] x 100

Where, As and Ac are the absorbance of the sample and control, respectively.

Ferric thiocyanate (FTC) assay: The FTC assay was carried out as described in the method of Osawa and Namiki [22]. Percentage of antioxidant activity was calculated using the following equation:

AA% = [($A_c - A_s$)/A_c] x 100

Where AA is antioxidant activity, A_c and A_s are the max absorbance values for control and samples, respectively.

Ferric reducing antioxidant power assay: The Ferric reducing antioxidant power assay was carried out as described in the method of Benzie and Strain [23]. The principle of this assay is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, coloured form in the presence of antioxidants. The change in absorbance between final reading selected and the blank reading was calculated for each of the oil samples and related to absorbance of a Fe^{+2} standard solution.

The FRAP value (mmol/l) =

[$_{(0-4\ min)}\Delta A_{593}$ of test sample]/[$_{(0-4\ min)}\Delta A_{593}$ of standard] x [Fe^{2+} standard (mmol/l)]

Thiobarbituric acid (TBA) assay: The Thiobarbituric acid (TBA) assay was carried out as described in the method of Ottolenghi [24]. The formation of malonaldehyde is the basis for the well known TBA method used for evaluating the extent of lipid peroxidation. Percentage of antioxidant activity was calculated using the same equation that was used to calculate the antioxidant activity in the FTC method.

β-carotene-linoleate bleaching assay: The antioxidant activity of *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil was evaluated using the β-carotene bleaching method following modification of the procedure described by Velioglu *et al.* [25]. The antioxidant activity of each of the oil samples was calculated as percent inhibition relative to control using the following equation [26].

AA (%) = [1 – (A_0 -A_t) / ($^oA_0$ - ^oA_t)] x 100

where AA is antioxidant activity, A_0 and $^oA_0$ are the absorbance values measured at zero time of incubation for oil samples and control, respectively while A_t and ^oA_t are the absorbance for oil samples and control, respectively at t = 120 min.

Determination of EC_{50} values: The sample concentrations providing 50% of antioxidant activity or 0.5 of absorbance (EC50) were calculated from the graphs of antioxidant activity percentages (DPPH, FTC, FRAP, TBARS and β-carotene-linoleate bleaching assay) against sample concentrations. BHT was used as positive control.

In vitro Anti-diabetic Activity Essential oil samples of *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves were assessed for *in vitro* anti-diabetic activity by the α- amylase, glucose uptake by yeast cells and α-glucosidase inhibition.

α-amylase Inhibition Activity : The α-amylase inhibition assay was performed using the 3, 5-dinitrosalicylic acid (DNSA) [27] with some modifications. Starch solution (0.25% w/v) was prepared by stirring 0.125 g of tapioca powder in 50 mL of 20 mM sodium phosphate buffer containing 6.7mM sodium chloride at pH 6.9. One unit of α-amylase enzyme solution was prepared by mixing 0.0253 g of α-amylase in 100 mL of cold distillation water. *Pimenta diocia* (L.) Merr., *Pimenta racemosa* (Mill.) oils were dissolved in DMSO to give concentrations (0.5, 1.00, 2.00 mg/ml). The color reagent was prepared by mixing sodium potassium tartrate solution (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH) and 96 mM of 3, 5- dinitrosalicylic acid solution (0.44 g of 3,5-dinitrosalicylic acid in 20 mL of deionized water). One unit of α-amylase solution, *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil were mixed thoroughly in a tube and incubated for 15 min. Then 500 μL of the starch solution was added into each tube and incubated for 15 min. The reaction was terminated by addition of 500 μL DNSA reagent, placed in boiling water bath for 5 min. The mixture was cooled to ambient temperature, diluted with 5 mL distilled water, and the absorbance was measured at 540 nm using a visible spectrophotometer. The blank control of reaction showing 100% enzyme activity was conducted by replacing the essential oil with DMSO (1.0 mL). To eliminate the absorbance effect of essential oil, a blank solution was also used and the reaction was terminated by DNSA before adding the starch solution. Acarbose solution (diluted in DMSO to 80 – 400 μL/mL) was used as a positive control. The production of maltose will de-

crease with α-amylase inhibitory activity which will result in reduced absorbance intensity. The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the following equation:

% of α- amylase enzyme inhibitory activity

= 100 - [(Maltose)$_{sample}$ / (Maltose)$_{control}$] x 100

The IC_{50}, which is the concentration of the sample required to inhibit 50% of the enzyme was determined for each sample.

α-glucosidase Inhibition Activity: α-glucosidase inhibitory activity of both leaves essential oils was carried out according to method of Bachhawat et al., [28] with slight modifications. In a 96-well plate, reaction mixture containing 50μl phosphate buffer (50mM, pH= 6.8), 10 μl α-glucosidase (1U/ml) [SRL] and 20 μl of varying concentrations of both essential oils were pre-incubated at 37^{o}C for 15 min. Then 20μl p-nitrophenyl-α-D-Glucopyranoside (PNPG) (1mM) [SRL] was added as a substrate and incubated further at 37^{o}C for 30 min. The reaction was stopped by adding 50μl sodium carbonate (0.1M). The yellow color produced was read at 405 nm using visible spectrophotometer. Acarbose was included as a standard at various concentrations (0.5, 1.00 and 2.00 mg/ml). The control samples were prepared without any essential oil. The result is expressed as percentage inhibition, which was calculated as follow:

% inhibition = [(Ac – As)/ Ac] x 100

Where, Ac is the absorbance of the control reaction (containing all reagents except the essential oil test sample), and As is the absorbance of the test essential oil sample. Acarbose; is a well-known α-glucosidase and α-amylase inhibitor which was used as reference drug for the inhibitory activity.

The IC_{50}, which is the concentration of the sample required to inhibit 50% of the enzyme was determined for each sample.

Glucose Uptake by Yeast Cells: Yeast cells were prepared according to the method of Cirillo, [29]. Briefly, commercial baker's yeast was washed by repeated centrifugation (3,000 rpm; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of both essential oils (0.5, 1.00, 2.00 mg/ml) were added to 1 mL of glucose solution (5, 10 and 25mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 μl of yeast suspension, vortex and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 rpm; 5 min) and glucose was estimated in the supernatant by DNSA method.

Metronidazole was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

Inhibition% = [(As – Ac)/ As] x 100

Where, Ac is the absorbance of the control reaction (containing all reagents except the test oil sample), and As is the absorbance of the test essential oil sample. The amount of glucose lingering in the medium after a specific time serves as a marker of the glucose uptake by the yeast cells.

Statistical analysis

All experiments were carried out in three replicates and presented as mean $\pm$ standard deviation (SD). The data were statistically analyzed by one-way ANOVA. The level of statistical significance was set at $p < 0.05$.

Results & Discussion

Yield, Physical characters and GC-MS analysis of Essential oil:

The percentage yields of *Pimenta racemosa* (Mill.) and *Pimenta diocia* (L.) Merr. essential oil were (2.5 and 1.5% v/w) (calculated on dry weight basis) respectively; the refractive indices recorded at 20 ^{o}C were close ranging from (1.3667-1.3377); essential oil of *Pimenta racemosa* (Mill.) leaves acquired yellowish color while that of *Pimenta diocia* (L.) Merr. leaves was white in color, the specific gravity of both samples (determined at 25 ^{o}C) were 0.8 and 0.9 respectively; and they were readily soluble in ethanol (70%).

The GC/MS results of oil samples of the leaves of *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.), displayed in Tables (1&2), which revealed qualitative and quantitative variation in the chemical composition. *Pimenta racemosa* (Mill.) leaves essential oil contains 29 compounds were identified which accounted for 98.8% of the essential oil phenyl propanoid (eugenol) was the major constituent identified (37.95%). The monoterpene hydrocarbon was the most abundant fraction (48.42%) and the major *constituents* were β-myrecene (21.01%), α-pinene (17.82%) and limonene (5.93%); Oxygenated monoterpene linalool (6.15).

In *Pimenta diocia* (L.) Merr. leaves essential oil composition showed 38 compounds were identified by GC/MS analysis, representing 99.11% of total essential oil. Monoterpene hydrocarbons represent (41.85%) of total oil composition while oxygenated monoterpenes represent (20.08%). Eugenol (30.17%), limonene (17.24%), α-pinene (16.78%), linalool (9.71%), 1,8-cineole (8.31%) and β-myrecene (5.21%) were the major compounds.

GC/MS results of the leaves of *Pimenta racemosa* (Mill.) and *Pimenta diocia* (L.) Merr. revealed that major identified compounds were (eugenol & β-myrecene) and (eugenol & limonene) respectively. Our result comes in agreement with

some of the published literature which enumerated eugenol as one of the major components of the chemical composition of the leaves essential oil of *Pimenta* species from Jamaica revealing that phenyl propanoid eugenol ranging from (54.3- 79.2%) [30, 31]. *Pimenta jamaicensis* predominant leaf oil were eugenol (61.8%) and limonene (10.4%) [32]. Other literatures revealed that leaf oil of *Pimenta* spp. may be dominated by chavicol, 1,8-cineole, methyl chavicol, geraniol and myrcene [33, 34]. A study conducted by [35] revealed that major constituents identified in *Pimenta racemosa (Mill.)* var. *terebinthina* were α-terpineol acetate (27%), α-terpineol (20%) and 4-methoxy eugenol (12.6%).

Regarding to our study we can ascertain that the chemotype of the essential oil the leaves of *Pimenta racemosa* (Mill.) cultivated in Egypt, is rich in (eugenol & β-myrecene) whilst that of and *Pimenta diocia* (L.) Merr. is rich in (eugenol & limonene). The decrease in eugenol percentage may be attributed to genetic change in the plant or environmental conditions such as variation of geographical origin, soil type, growing conditions and harvest time [36].

Table 1 Chemical composition of the essential oil of the leaves of *Pimenta racemosa*(Mill.).

No.	RI*	Compound	%
1	800	hexenal	0.20
2	937	**α-pinene**	**17.82**
3	950	camphene	0.20
4	991	**β -myrecene**	**21.01**
5	1004	α-phellandrene	1.48
6	1015	α-terpinene	0.45
7	1025	p-cymene	0.78
8	1027	**limonene**	**5.93**
9	1032	1,8 cineole	1.25
10	1037	β -ocimene	0.75
11	1093	**linalool**	**6.15**
12	1170	α-terpineol	0.41
13	1215	citral	0.15
14	1229	nerol	0.22
15	1248	chaviol	0.80
16	1253	geraniol	0.33
17	1365	**eugenol**	**37.95**
18	1369	methyl eugenol	0.30
19	1418	β-caryophyllene	0.25
20	1454	α -humulene	0.14
21	1472	muurolene	0.15
22	1482	germacrene D	0.17
23	1498	α-farnesene	0.15
24	1522	δ -cadinene	0.60
25	1528	elemicine	0.20
26	1572	spathulenol	0.15
27	1575	caryophyllene oxide	0.34
28	1630	α-muurolol	0.27
29	1643	α-cadinol	0.20
Total identified percentage			98.8
Monterpene Hydrocarbons			48.42
Phenyl propanoid			39.25
Oxygenated Monoterpens			8.51

In-vitro antioxidant activity

Antioxidant activities of the essential oils from aromatic plants are mainly attributed to the active compounds present in them. This may be due to the high percentage of main constituents, but also may be due to the presence of other constituents present in small quantities [37, 38] . DPPH radical assay is the simplest method to measure the ability of antioxidants and based on the transfer of electrons from a donor molecule to the corresponding radical [39]. Results of the present study showed the scavenging activity of *Pimenta diocia* and *racemosa* essential oil were significantly lower (92.7%, 63.8 %, respectively) when compared to BHT (90.2%) table (3).

The primary stage of linoleic acid peroxidation is measured using FTC method. As the antioxidant activity increases, the peroxide concentration decreases. Malonaldehyde (MDA) is a by-product of lipid peroxidation that is formed from the oxidation of linoleic acid. The antioxidant activity of both oil samples has markedly inhibited the oxidation of linoleic acid when compared to the control. This present data indicates that *Pimenta diocia* and *racemosa* essential oils showed a pronounced antioxidant activity but less than BHT in stabilizing linoleic acid.

The reducing power of antioxidants measured using FRAP assay [40]. It measures the reduction of (Fe^{3+}) ion to (Fe^{2+}) ion in the presence of antioxidants. Results showed that *Pimenta diocia* and *racemosa* essential oil had higher FRAP capacity compared to BHT table (3). The order of antioxidant effectiveness or reducing effect power was: *Pimenta diocia* (L.) Merr. > *Pimenta racemosa* (Mill.) > BHT. Free radicals which produced by iron may cause DNA double strand destroying and maintain the growth of malignant cells as well as the growth of the pathogen. On the other hand iron chelators may protect the cell against damage. The present mechanism was confirmed with Olivera et al., [41], which shows that the essential oil may lower the incidence of certain cancer diseases.

Eugenol the major identified in the oil analysis has many medicinal uses, as it has a monohydroxy substitution in the aromatic ring that possesses hydrogen donating ability and may participate in this activity [42].

In vitro Anti-diabetic Activity

α-amylase Inhibition Activity of Pimenta diocia (L.) Merr. & Pimenta racemosa (Mill.) leaves essential oil: The alpha amylase activity of *Pimenta racemosa* (Mill.) leaves essential oil is greater than the alpha amylase activity of *Pimenta diocia* (L.) Merr. leaves essential oil. The gradual increase in the percentage of α-amylase inhibition activity when the concentration is in-

Table 2 Chemical composition of the essential oil of the leaves of *Pimenta dioica*(L.) Merr.

No.	RI*	Compound	%
1	860	hexenal	0.17
2	930	α-thujene	0.30
3	941	**α-pinene**	**16.78**
4	981	sabiene	1.04
5	994	**β-myercene**	**5.21**
6	1010	α-phellandrene	0.40
7	1019	δ-carene	0.11
8	1025	p-cymene	0.30
9	1027	**limonene**	**17.24**
10	1040	**1,8- cineol**	**8.31**
11	1050	β-ocimene	0.12
12	1057	γ-terpinene	0.15
13	1086	terpinolene	0.20
14	1090	**linalool**	**9.71**
15	1189	Terpinen-4-ol	0.13
16	1200	α-terpineol	1.93
17	1260	chavicol	0.60
18	1383	**eugenol**	**30.17**
19	1390	α-copaene	0.23
20	1395	β-elemene	0.43
21	1402	methyl eugenol	0.98
22	1418	β-caryophyllene	0.61
23	1436	γ-elemene	0.39
24	1438	aromadendrene	0.11
25	1449	β-eudesmol	0.10
26	1454	α-humulene	0.31
27	1477	γ-gurjunene	0.10
28	1481	alloaromadendrene	0.37
29	1500	α-muurolene	0.20
30	1504	germacerene D	0.62
31	1508	α-farnesene	0.10
32	1519	α-selinene	0.15
33	1522	δ -cadinene	0.83
34	1582	caryophyllene-oxide	0.15
35	1630	γ-eudesmol	0.11
36	1642	α-muurolol	0.20
37	1653	α-cadinol	0.15
38	1741	farnesal	0.10
Total identified percentage			99.11
Monoterpens Hydrocarbons			41.85
Oxygenated Monoterpens			20.08
Phenyl propanoid			31.75
Sesquiterpens Hydrocabon			

*=Calculated retention index

creased from 0.5 to 2.00 mg and when the concentration at 2.00 mg, the percentage of α- amylase activity of *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) oil is 75 and 63.90%, respectively Table (4).

Thus, data presented here indicates that *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil possesses significant *in vitro* antidiabetic activity. The mechanism by which essential oil of both *Pimenta* species exerted this action may be due to its action on carbohydrate binding regions of α- glucosidase enzyme, α- amylase, endoglucanases that catalyse hydrolysis of the internal α-1,4 glucosidic linkages in starch and other related polysaccharides have also been targets for the suppression of postprandial hyperglycemia**.** This enzyme is responsible in hydrolyzing dietary starch into maltose which then breaks down to glucose prior to absorption. Since α-amylases play an important role in starch break down in human beings and animals, the presence of such inhibitors in food stuffs may be responsible for impaired starch digestion [43, 44]. α-amylase inhibitor may be of value as a good therapeutic dietetic agents. In conclusion, data presented here rationalize that *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil have potential to emerge as new remedy for treatment of type-II diabetes mellitus.

Acarbose-like drugs, that inhibit α- glucosidase present in the epithelium of small intestine, have been demonstrated to decrease postprandial hyperglycemia [45] and improve impaired glucose metabolism without promoting insulin secretion in NIDDM patients [46].

α- *Glucosidase Inhibition Activity:* The percentage of α-glucosidase activity of *Pimenta racemosa* (Mill.) oil is slightly greater than that of *Pimenta diocia* (L.) Merr. oil. The gradual increase in the percentage of α-glucosidase activity, when concentration is increased from 0.5 to 2.00 mg. When the concentration at 2.00 mg, the percentage of α- glucosidase activity of *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) oil is (61.42%) and (53.00%); respectively, results displayed in Table (5).

The present study is the first report on that α- glucosidase inhibitory effect of *Pimenta racemosa* (Mill.) and *Pimenta diocia* (L.) Merr. leaves essential oil.

Glucose Uptake by Yeast Cell: The rate of uptake of glucose into yeast cells was linear in all the 3 glucose concentrations. The percentage of glucose uptake by *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil in 5 mM is 27.59% and 17.44%, respectively Table (6). The gradual increase in the percentage of glucose uptake by *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil in 5 mM when the concentration is increased from 0.5 to 1.00 mg. When the concentration at 2.00 mg, the percentage of Glucose uptake by *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil in 10 mM is (41.30%) and (39.77 %); respectively. The gradual increase in the percentage of glucose uptake by *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil in 10 mM when the concentration is increased from 0.5 to 1.00 mg. *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) oil in 25 mM when the concentration is increased from 0.5 to 1.00 mg. When the concentration at 2.00 mg, the percentage of glucose uptake by *Pimenta diocia* (L.) Merr. and *Pimenta*

Table 3 Antioxidant activity of *Pimenta diocia* and *Pimenta racemosa* leaves essential oils using different methods: DPPH, FTC, FRAP, TBARS and β-carotene-linoleate bleaching assay.

Samples	Antioxidant activity									
	DPPH		FTC		FRAP (mmol / L)		TBARS		β-carotene-linoleate bleaching assay	
	%	EC_{50}	%	EC_{50}	%	EC_{50}	%	EC_{50}	%	EC_{50}
Pimenta diocia	92.7	0.65 ± 0.4^{a}	85.7	1.1 ± 0.1^{a}	3.1	6.11 ± 0.3^{a}	76.7	2.68 ± 0.08^{a}	96.8	0.47 ± 0.3^{a}
Pimenta racemosa	63.8	3.8 ± 0.3^{c}	75.3	2.9 ± 0.07^{c}	2.4	12.54 ± 0.2^{c}	62.11	3.92 ± 0.05^{c}	94.5	0.54 ± 0.3^{c}
BHT	90.2	0.97 ± 0.3^{b}	63.4	3.8 ± 0.05^{b}	1.5	9.36 ± 0.5^{b}	51.5	3.98 ± 0.07^{b}	105.8	0.22 ± 0.3^{b}

The antioxidant activity was expressed as percent(%) and EC50 values (mean $\pm$ SD), what means that higher values correspond to lower reducing power or antioxidant potential. EC50: essential oils concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance in reducing power assay. In each row different letters mean significant differences between *Pimenta diocia* and *Pimenta racemosa* essential oils ($p<0.05$).

Table 4 % Enzyme Relative activity and IC_{50}values (mg/mL) for *Pimenta diocia* & *Pimenta racemosa* leaves essential oil and Acarbose in α-amylase inhibitory assay

Conc. (mg/ml)	% Enzyme Relative activity ***Pimenta diocia***	% Enzyme Relative activity ***Pimenta racemosa***	% Enzyme Relative activity **Acarbose**
0.5	25.15 ± 0.50	20.55 ± 0.78	20.50 ± 0.80
1.00	45.09 ± 0.60	40.60 ± 0.80	36.70 ± 0.90
2.00	75.00 ± 0.90	63.90 ± 0.50	55.86 ± 1.10
	IC_{50} (mg/dl)= 0.95 ± 0.10^{c}	IC_{50} (mg/dl)= 1.13 ± 0.190^{b}	IC_{50} (mg/dl)= 1.75 ± 0.03^{a}

Values are the mean of triplicate experiments and represented as mean$\pm$SEM (n=3). Values in the same column with different superscripts are significantly different ($P<0.05$). Student's t test was performed to analyze this data set.

Table 5 % Enzyme Relative activity and IC_{50}values (mg/mL) for *Pimenta diocia* & *Pimenta racemosa* leaves essential oil and Acarbose in α-glucosidase inhibitory assay.

Conc. (mg/ml)	% Enzyme Relative activity ***Pimenta diocia***	% Enzyme Relative activity ***Pimenta racemosa***	% Enzyme Relative activity **Acarbose**
0.5	37.06 ± 0.24	30.50 ± 0.31	28.09 ± 0.06
1.00	54.11 ± 0.38	39.26 ± 0.40	33.00 ± 0.25
2.00	61.42 ± 0.50	53.00 ± 0.33	42.62 ± 0.61
	IC_{50} (mg/dl)= $3.17\pm0.0.6^{c}$	IC_{50} (mg/dl)= 4.25 ± 0.080^{b}	IC_{50} (mg/dl)= 5.28 ± 0.05^{a}

Values were expressed as mean $\pm$ SE (n = 3). The different letters indicate a significant difference between the oils ($p < 0.05$). IC50 (mg/mL): the concentration at which 50% is inhibited.

racemosa (Mill.) oil in 25 mM is (63.49%) and (49.61%); respectively, results are shown in Table (6).

The mechanism of glucose transport across the yeast cell membrane has been receiving attention as an important method for in vitro screening of hypoglycemic effect of various compounds/ medicinal plants. It was observed that both the *Pimenta* diocia (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil promoted glucose transport across the yeast cells. The rate of uptake of glucose into the yeast cells was linear in all the 3 glucose concentrations (0.5, 1.0, 2.0 mg/ml) used in the study. Several studies on the transport of non metabolizable sugars, metabolizable glycosides have suggested that sugar transport across the yeast cell membrane is mediated by stereospecific membrane carriers and usually takes place by the process of facilitated diffusion [47]. The antihyperglycemic action of the *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil may be due to blocking of glucose absorption [48]. Eugenol has been reported for its antidiabetic activity [49], also limonene one of the major identified constituent in both *Pimenta* species leaves essential oil species has been reported to show anti-diabetic activity [50, 51]. The GC/MS analysis of the essential oil of both *Pimenta* species analysis in the present study proved the presence of various phytoconstituents namely α-pinene, β -myrecene, α-phellandrene, β -ocimene, α -humulene, δ -cadinene, etc. which may contribute towards the said antidiabetic activity [52]. Morever *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil being a commonly used as an ethnomedicinal drug by various sects of tribal communities in Egypt and the powder clove of the seed are taken with water to lower the blood sugar level hence the study was directing towards the activity of *Pimenta*.

Table 6 % of uptake of glucose for Pimenta diociaand Pimenta racemosa leaves essential oil

		Pimenta diocia (1 mL glucose, mM)			Pimenta racemosa (1 mL glucose, mM)			% uptake of glucose ***Pimenta diocia***			% uptake of glucose ***Pimenta racemosa***		
Conc. (mg/ml)	Con-trol	5	10	25	5	10	25	5	10	25	5	10	25
0.5	0.530 ±0.00	0.732 ±0.04	0.802 ±0.02	0.980 ±0.06	0.642 ±0.02	0.700 ±0.04	0.902 ±0.02	27.59 ±2.04 ^{Aa}	33.91 ±2.54^{Aa}	45.91 ±1.97^{Aa}	17.44 ±1.93^{Aa}	24.28 ±2.90^{Aa}	41.24 ±2.73^{Aa}
1.00	0.530 ±0.00	0.770 ±0.04	0.855 ±0.05	1.203 ±0.07	0.690 ±0.03	0.832 ±0.06	0.953 ±0.03	31.16 ±1.05^{a}	38.01 ±3.98	55.94 ±3.56^{Aa}	23.18 ±1.45^{a}	36.29 ±3.05^{Aa}	44.38 ±2.07^{a}
2.00	0.530 ±0.00	0.832 ±0.03	0.903 ±0.02	1.452 ±0.04	0.731 ±0.06	0.880 ±0.03	1.052 ±0.008	36.29 ±3.04^{Aa}	41.30 ±2.76^{A}	63.49 ±4.09^{Aa}	27.49 ±2.87^{Aa}	39.77 ±2.25^{Aa}	49.61 ±1.63^{Aa}

Values were expressed as mean ± SE (n = 3). The different letters indicate a significant difference between the oils (p < 0.05). A: Means that the values of % uptake of glucose for *Pimenta diocia* and *Pimenta racemosa* essential oil in the same column are significantly at (P<0.05).
a: Means that the values of % uptake of glucose for pimenta diocia and pimentaracemosa essential oil in the same raw are significantly at(P<0.05).

Conclusion

In this study we declared that the chemotype of the essential oil of the leaves of *Pimentaracemosa* (Mill.) cultivated in Egypt, is rich in (eugenol & β-myrecene) while that of and *Pimenta diocia* (L.) Merr. is rich in (eugenol & limonene). The antioxidant activity of the leaves essential oil of both *Pimenta* species may be attributed to high amount of eugenol. This study also shows that both leaves essential oil can be used as a potential source of natural antioxidants, with possible application in food system. The present work reported for the first time the elevation of glucose uptake by yeast cell as well as α-amylase and α-glucosidase inhibitory effect of both *Pimenta* leaves essential oil. The results indicated that the both oils are promising hypoglycemic agent and can be included in antidiabetic preparations. The hypoglycemic activity of the oil is mainly due to its high content of various phytoconstituents. This is the first document of antioxidant and antidiabetic activities of the essential oil of both *Pimenta* species under study. Since interest in drugs of herbal origin has significantly increased, further in vivo studies are needed to confirm the hypoglycemic effect of the *Pimenta diocia* (L.) Merr. and *Pimenta racemosa* (Mill.) leaves essential oil alone and in combination with acarbose.

Conflict of interest

The authors declare no conflict of interest.

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Author details

[1]Pharmacognosy Department, Faculty of Pharmacy, October 6 University, Sixth of October City, 12585, Egypt. [2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Future University, Egypt. [3]Department of Pharmacology and Toxicology,, College of Pharmacy, Misr University for Science and Technology (MUST), October 6th City, 12585, Egypt. [4]Biochemistry Department, Faculty of Applied Medical Sciences, October 6 University, Sixth of October City, 12585, Egypt. Tel.: +2(0122) 4832580, Fax: +2 (02) 38353270.

References

[1] Bailey LH, Manual of cultivated Plants. New York: The Macmillan Company; 1968.

[2] Charles D, Simon J. Comparison of extraction methods for the rapid determination of essential oil content and composition of Basil. Journal of the American Society for Horticultural Science. 1990;115:458–462.

[3] Kapoor IPS. Chemistry and in vitro antioxidant activity of volatile oil and oleoresins of black pepper (Piper nigrum). Journal of Agricultural and Food Chemistry. 2009;57:5358–5364.

[4] Leaf essential oil composition of Pimenta pseudocaryophyllus (Gomes) L. R. Landrum native from Brazil. Marques FA, Wendler EP, Baroni ACM, de Oliveira PR, Sasaki BS, Guerrero J. 2010;22(2):150–152.

[5] Rao PS, Navinchandra S, Jayaveera KN, "An important spice, Pimenta dioica (Linn.) Merill: A review". International Current Pharmaceutical Journal. 2012;1(8):221–225.

[6] Paula JAM, Reis JB, Ferreira LHM, Menezes ACS, Paula JR. "Gênero Pimenta: aspectos botânicos, composição química e potencial farmacológico". Revista Brasileira de Plantas Medicinais. 2010;12(3):363–379.

[7] Weiss EA. Spice Crops. New York: CABI Publishing; 2002.

[8] Boning CR, "Native and Exotic Plants Grown for Scent and Flavor". Florida's Best Herbs and Spices. Florida:

Pineapple Press Inc., 1st; 2010.

[9] Contreras-Moreno B, Rojas J, Celis M, Rojas L, Méndez L, Landrum L. "Componentes volátiles de las hojas de Pimenta racemosa (Mill.) var. racemosa (Mill.) JW Moore (Myrtaceae) de Táchira–Venezuela". Bol Latinoam Caribe Plant Med Aromat. 2014;13(3):305–310.

[10] Jirovetz L, Buchbauer G, Stoilova I, Krastanov A, Stoyanova A. "Spice Plants: Chemical Composition and Antioxidant Properties of Pimenta Lindl. Essential Oils. Part 1: Pimenta dioica (L.) Merr. Leaf oil from Jamaica". Ernaehrung. NUTRITION-VIENNA. 2007;31(2):55–62.

[11] Alitonou GA, Noudogbessi JP, Sessou P, Tonouhewa A, Avlessi F, Menut C, Sohounhloue DC "Chemical composition and biological activities of essential oils of Pimenta racemosa (Mill.) from Benin". Int J Biosci. 2012;2:1–12.

[12] Leyva MI, Tacoronte JE, Navarro A, Montada D, Bello A, Marquetti MC "Estudio de laboratorio del aceite escencial de Pimenta racemosa (Myrtales: Myrtaceae) y su posible utilización para el control de Musca domestica (Diptera: Muscidae)". Rev CENIC Ciencias Biológicas. 2007;38(1):18–19.

[13] Tajkarimi MM, Ibrahim SA, Cliver DO "Antimicrobial herb and spice compounds in food". Food Control. 2010;21(9):1199–1218.

[14] Kim J, Lee YS, Lee SG, Shin SC, Park IK. Fumigant antifungal activity of plant essential oils and components from West Indian bay (Pimenta racemosa (Mill.)) and thyme (Thymus vulgaris) oils against two phytopathogenic fungi. Flavour Fragr J. 2008;23(4):272–277.

[15] Nesrin S, Sevil O, Mahmut G, Kerim G, Osman EO, Moustafa MK "Determination of antimicrobial activity and chemical composition of pimento & ginger essential oil". Indian J Pharm Educ Res. 2017;51(3):230–233.

[16] El-Gizawy HA, Hussein MA. "Fatty acids Profile, Nutritional Values, Anti-diabetic and Antioxidant Activity of the Fixed Oil of Malva parviflora growing in Egypt". International journal of Phytomedicine. 2015;7(2):219–230.

[17] Patel DK, Kumar R, Prasad SK, Sairam K, Hemalatha S. Antidiabetic and in vitro antioxidant potential of Hybanthus enneaspermus(Linn) F. Muell in streptozotocin induced diabetic rats. Asian Pac J Trop Biomed. 2011;1(4):316–322.

[18] Malviya N, Jain S, Malviya S. Anti-diabetic potential of medicinal plants. Acta Pol Pharm. 2010;67(2):118.

[19] Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK. "Antidiabetic agents from medicinal plants". Curr Med Chem. 2006;13(10):1203–1218.

[20] Ponnusamy S, Ravindran R, Zinjarde S, Bhargava S, Kumar AR. Evaluation of traditional Indian anti-diabetic medicinal plants for human pancreatic amylase inhibitory effect in vitro. Evidence Based Complement Alternat Med. 2011;51(5):647–653.

[21] Shimada K, Fujikawa K, Yahara K, undefined Nakamura. T "Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion". J Agric Food Chem. 1992;40(6):945–948.

[22] Osawa T, Namiki T "A novel type of antioxidant isolated from leaf wax of Eucalyptus leaves". J Agric Food Chem. 1981;45(3):735–739.

[23] Benzie IFF, Strain JJ. "The ferric reducing ability of plasma (FRAP) as a measure of " antioxidant power : The FRAP assay". Anal Biochem. 1996;239(1):70–76.

[24] Ottolenghi A "Thiobarbituric acid (TBA) method". Arch Biochem Biophys. 1959;77:355.

[25] Velioglu YS, Mazza G, Gao L, Oomah BD. "Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products". J Agric Food Chem. 1998;46(10):4113–4117.

[26] Jayaprakasha GK, Sign RP, Sakariah KK "Antioxidant activity of grape seed (Vitis vinifera) extracts on peroxiation models in vitro". Food Chem. 2001;73(3):285–290.

[27] L MG. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 1959;31(3):426–428.

[28] Bachhawat A, Shihabudeen MS, Thirumurugan K. "Screening of fifteen Indian Ayurvedic plants for α- glucosidase inhibitory activity and enzyme kinetics". International Journal of Pharmacy and Pharmaceutical Sciences. 2011;3(4):267–274.

[29] Cirillo VP. "Mechanism of Glucose Transport across the Yeast Cell Membrane". J Bacteriol. 1962;84(3):485–491.

[30] Tucker AO, Maciarello MJ, Landrum LR. "Volatile Leaf Oils of Caribbean Myrtaceae. II. Pimenta dioica (L.) Merr. of Jamaica" Journal of Essential Oil Research. 1991;3(3):195–196.

[31] Pino JA, Rosado A. "Chemical Compostion of the Leaf Oil of Pimenta dioica L. from Cuba". Journal of Essential Oil Research. 1996;8(3):331–332.

[32] Tucker AO, Maciarello MJ, Landrum LR "Volatile leaf oils of Caribbean Myrtaceae. IV. Pimenta jamaicensis (Britton. & Harris) Proctor of Jamaica" J Essent Oil Res. 1992;4(1):93–94.

[33] Tucker AO, Maciarello MJ, Adams RP, Landrum LR, Zanoni TA. "Volatile Leaf Oils of Caribbean Myrtaceae. I. Three varieties of Pimenta racemosa. Journal of Essential Oil Research. 1991;3(5):323–329.

[34] Bello A, Rodriguez ML., Castineira N, Urquiola A, Rosado A, Pino JA "Chemical Composition of the Leaf Oil

of Pimenta racemosa (Mill.) J.Moore from Western Cuba". Journal of Essential Oil Research. 1995;7(4):423–424.

[35] Dolores G, Antonio A, Paz T, Angeles F, Teresa S. "Gas Chromatographic-Mass Spectrometry Study of the Essential Oils of Pimenta racemosa (Mill.) var. terbinthina and P. racemosa var. grisea" Verlag der Zeitschrift fur Naturforschung. vol. 57 of 5-6. Tubingen; 2002. p. 449–451.

[36] Yoshimura M, Amakura Y, Yoshida T. "Polyphenolic Compounds in Clove and Pimento and their Antioxidative Activities". Biosci Biotech and Biochem J. 2011;75(11):2207–2212.

[37] El-gizawy HA and Hussein MA. "Isolation, Structure Elucidation of Ferulic and Coumaric acids from Fortunella japonica Swingle leaves and their Structure Antioxidant activity relationship". Free radical and Antioxidants. 2017;7(1):23–30.

[38] Synergistic effect of Thymol and carvacrol combined with chelators and organic acids against Salmonella Typhimurium. J Food Prot. 2007;70(7):1704–1709.

[39] Hussein MA. A Convenient Mechanism for the Free Radical Scavenging Activity of Resveratrol. International Journal of Phytomedicine. 2011;3(4):459–469.

[40] Hussein MA, El-gizawy HA, Gobba NA, Mosaad YO. "Synthesis of Cinnamyl and Caffeoyl Derivatives of Cucurbitacin-Eglycoside Isolated from Citrullus colocynthis Fruits and their Structures Antioxidant and Anti-inflammatory Activities Relationship". Current Pharmaceutical Biotechnology. 2017;18(8):677–693.

[41] Olivera P, Mladen MJ, undefined M. Chemical Composition and Antioxidant Activity of Essential Oils of Twelve Spice Plants. Croatica Chemica. 2006;79(4):545–552.

[42] Gulcin I, "Antioxidant activity of eugenol: a structure-activity relationship study". Journal of Medicinal Food. 2011;14(9):975–985.

[43] Marshall JJ. Hypothesized that negatively charged residues of pilaic acid from membrane. In: and others, editor. Am Chem Soc Symposium Series. vol. 15; 1975. p. 244–66.

[44] Jaffe WG, Lette CLV. "Heat labile growth inhibiting factors in bens (Phaseolus vulgaris)". J Nutr. 1968;94(2):203–10.

[45] Sima AAF, Chakrabarti S. Long term suppression of post prandial hyperglycemia with acarbose retards the development of neuropathies in the BB/W-rat. Diabetologia. 2004;35:325–30.

[46] Carrascosa JM, Molero JC, Fermin Y, Martinez C, Andres A, Satrustegui J. "Effect of chronic treatment with acarbose on glucose and lipid metabolism in obese diabetic wistar rats". Diabetes Obes Metab. 2001;3(4):240–248.

[47] Teusink B, Diderich JA, Westerhoff HV, Van DK, Walsh MC. Intracellular glucose concentration in derepressed yeast cells consuming glucose is high enough to reduce the glucose transport rate by 50%. J Bacteriol. 1998;180(3):556–562.

[48] Kannur DM, Hukkeri VI. and Akki KS. Antidiabetic activity of Caesalpinia bonducella seed extracts in rats. Fitoterapia. 2006;77(7-8):546–549.

[49] Pyiyanka S, Ramesha H, Sachin B, Garikapati V, Arvind M, Atul A, Vihal S, Mahemud L, Rakesh S, Ramanamurthy B, Mahesh J, Hirekodathakallu V, Ashok p. "Potential dual role of eugenol in inhibiting advanced glycation end products in diabetes: proteomic and mechanistic insights". Scientific Reports. 2016;6:18798.

[50] Murali R, Karthikeyan A, Saravanan R "Protective effects of D-limonene on lipid peroxidation and antioxidant enzymes in streptozotocin- induced diabetic rats". Basic & Clin Pharmacol & Toxicol. 2013;112:3–175.

[51] More TA, Kulkarni BR, Nalawade ML and Arvindekar AU. "Antidiabetic activity of linalool and limonene in streptozotocin-induced diabetic rat: a combinatorial therapy approach,". International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(8):159–163.

[52] Shukla S, Mehta P, Mehta A, Vyas SP, Bajpai VK. Preliminary phytochemical and antifungal screening of various organic extracts of Caesalpinia bonducella seeds. Romanian Biotechnological Letters. 2011;16(4):6384–6389.