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In-vitro Pharmacological Activities of Mango *(Mangifera Indica)* and Olive (*Olea Europaea)* leaves

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ABSTRACT

The prevalence of obesity, diabetes mellitus, albumin denaturation as well as it's downstream inflammation-based diseases had become a global health problem. In addition, the increased incidence of Helicobacter pylori (*H. pylori)* infection raises the demand for new approaches to tackle these complications using natural, plant-based materials. Natural plant secondary metabolites had been shown to have anti-inflammatory (albumin denaturation and anti-histamine), anti-diabetic and *H. pylori* effects. This study intended to extract and characterize the bioactive compounds from the ethanol extract of Mango (*Mangifera indica)* leaf (MIL) and Olive (*Olea europaea)* leaf (OEL) using defined HPLC methods. The study is evaluating the anti-inflammatory effects of these extracts as well as its inhibitory influences against *H. pyroli* growth. The results revealed that quercitrin and kaempferol were found in high concentrations in both mango and olive extracts (8.61 and 9.05 µg/mg, respectively). MIL showed a significant effect of histamine release inhibition and diabetic action (76.19 % and 71.60 % respectively). However, the IC_{50} values of α-glucosidase inhibition indicated that MIL has inhibition capabilities near to acarbose, and higher than OEL (30.57, 71.6 and 129.0 for acarbose, mango, and olive extracts respectively). Furthermore, OEL presented a significant effect against albumin denaturation, the IC_{50} was 44.02% while MIL showed 448.6% inhibition of protein denaturation. Moreover, OEL exhibited an anti-*H. Pylori* activity with MIC value of 0.7, 92.61 and 6.6 µg/mL, for clarithromycin, mango and olive extracts respectively.

Practical application

Olea europaea and *Mangifera indica* leaves could be considered as cheap and plentiful raw materials that are rich in phenolic compounds in general and polyphenols specifically. I hypothesize the applicability of these materials as an anti-inflammatory (anti-albumin denaturation, antihistamine) and anti-diabetic agents. In addition, ethanolic leaves extracts showed an inhibitory effect against *H. pyroli* growth and activity. The current study suggests the involvement of these substances in food products and/or in the plant for health dietary supplements.

Keywords: Anti-Inflammatory, Anti-diabetic, Helicobacter pyroli, HPLC, Antihistamine

Introduction

Mango (*Mangifera indica* L.) is a standout amongst the most financially vital tropical plants (Barreto *et al.,* 2008), which belongs to the family Anacardiaceae. Currently, Mango leaves are getting more attention in the plant for health research because of its benefits in cell reinforcement and its antimicrobial and anticancer properties (Shah *et al.,* 2010). Mango is considered a rich source for different polyphenolic compounds, mainly mangiferin, which is the most significant compound that can be distinguished in all parts of the mango. This compound is a xanthone subsidiary that alluded as super cancer prevention agent Sekar, (2015) reported strong pharmacological influences of xanthone including cell reinforcement, radioprotective, antiallergic, antidiabetic, anticancer, antimicrobial, immunomodulatory, and anti-inflammatory activities. Mango leaves contain several phytochemical compounds such as; saponins, alkaloids, phenols, tannins, and flavonoids (Jhaumeer *et al.,* 2018). Medicinal value of *Mangifera indica* leaves had been used for centuries for the treatment of different kinds of diseases (Shah *et al.,* 2010). Similarly, these leaves are found to hold different pharmacological properties including the antibacterial activity (Doughari and Manzara, 2008).

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OEL contains an extensive variety of phenolic compounds, for example, oleuropein (OE), hydroxytyrosol (HT), tyrosol (T), cumaric acid, ferulic acid, and caffeic acid. These phenolic compounds had been shown to exhibit ant oxidation activities (Yuan *et al.,* 2015), and known with its high cell reinforcement, antimicrobial and antibacterial action. OLE showed a therapeutic effect on different diseases, such as diabetes and bacterial/ yeast contaminations (Vane, 1971).

Different parts of olive such as leaves, or fruits had been used broadly in traditional medicine. Olive leaves are found to be rich in polyphenols which are strong antioxidants and potent antiviral compounds (Caponio *et al.,* 2001). Previous studies on animals and humans have reported beneficial health effects of olive leaves as anti-hypertension and anti-hypercholesterolemia agents as well as coadjuvant in the treatment of obesity (Esmaeili-Mahani *et al.,* 2010). Hence, the current work aims to define the extracted compounds and further investigate the anti-inflammatory, anti-diabetic activities of *Mangifera indica* and *Olea europaea* leave extracts as well as their inhibitory effects against *H. pyroli*.

Material and Methods

Plant material

Mangifera indica (MI) and *Olea europaea* (OE) leaves were gathered from Salhia farms, Sharkia, Egypt. The leaves were dried for five days at room temperature (20-30°C). Afterward, the dried leaves were grinded into fine powder.

Preparation of MIL and OEL extract

The dried powder of each plant sample was extracted by maceration with 70 % ethanol (90 g dried powder into 900 mL of 70 % ethanol) for one day at room temperature. After stirring, the recovered filtrate was dried in a rotary evaporator for 40 minutes at 40°C. The yield of concentrate was then lyophilized and stored at - 40°C in closed containers until required according

HPLC Analyses of MIL and OEL

HPLC analyses were performed on HPLC (Agilent Technologies, Waldbronn, Germany) apparatus with a vacuum degasser, autosampler, a binary pump GBC LC 1110, and a detector GBC UV/ vis was used for the chromatographic determination. Phenolic and flavonoids components were isolated by utilizing KROMASIL column (4.6×150 mm, 1.8mm particle size) (Berek and Tarbajovská, 2002) operating at 25°C and a flow rate of 0.8 mL/min. and 1 mL/min., respectively) the mobile phases used were methanol : water : tetrahydrofuran with acetic acid (23:75:1:1 %) (Phase A) and acetonitrile (phase B) for phenolic components and acetonitrile: water: formic acid (85:14:1) (phase A) for flavonoids compounds the separated components were monitored in sequence first with GBC U.V/ vis at 280 and 356.

Anti-Inflammatory activity of MIL and OEL extract

Albumin denaturation assay

The activity was performed using albumin denaturation according to the method of Singh and Sharma, (2015) with few modifications. Two-fold serial dilutions of samples ranging from 7.81 -1000 µg/mL were prepared in 96-well plate. Response blend for every fixation was readied which comprised of 100 µL of test drug (diclofenac sodium standard (DSS)) and 100 µL of 1% bovine albumin solution. These prepared solutions were incubated at 28°C for 15 min. Then, the reaction mixtures were kept at 70°C for 10 min to induce denaturation. The mixture was cooled down and turbidity was estimated utilizing microplate peruse at 660 nm. Diclofenac sodium was utilized as a standard drug in the grouping of 7.81, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 µg/mL and regarded also as test removes. Rate hindrance of denaturation was computed utilizing control in which

no drug was included. Each trial was done in triplicate and the mean was taken**.** The rate restraint of protein denaturation was ascertained by the following equation.

% Inhibition of protein denaturation = 1-[A1/A2]100

Where: A1 = Absorbance of control

A2 = Absorbance of test/standard example with albumin arrangement. The IC_{50} esteem was characterized as the fixation to repress half of the protein denaturation under the measurement conditions.

Histamine Release Assay

937 human monocytes (ATCC, Manassas, VA, USA) were utilized to consider the impact of tests on histamine release. Roughly 50,000 U937 cells were plated in a 96-well cell culture plate (Corning Life Sciences, Lowell, MA, USA) and treated with different concentration (1000-7.81 µg/mL) of tests, in nearness or nonappearance of 20 nM Phorbol Myristate Acetate (PMA) (Sigma-Aldrich, St. Luis, MO, USA) for one h. The cell culture supernatants assembled from either untreated control or treated societies were explained at 10,000 g for 5 min at 4°C and assessed for released histamine by a monetarily open EIA unit (SPI-Bio, France) Diclofenac was used as a positive control.

Anti-diabetic activity of MIL and OEL (α-glucosidase inhibitory)

The α-glucosidase inhibitory activity was done by the standard strategy with a minor change (Shai *et al.,* 2011). In a 96-well plate, response blend containing 50 µL phosphate support (100 mM, pH = 6. 8), 10 µL α-glucosidase (1 U/mL), and 20 µL of shifting groupings of concentrates and divisions (1000 to 7.81 µg/mL) was pre-incubated at 37°C for 15 min. Then, 20 µL P-nitro-phenyl-α-D-glucopyranoside (P-NPG) (5mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was stopped by adding 50 µL Na_2CO_3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using a multiplate reader. Acarbose at various concentrations (1000 to 7.81 µg/mL) was included as a standard. Without a test, the substance was set up in parallel as a control and each investigation was performed in triplicates. The outcomes were communicated as rate restraint, which was ascertained utilizing the recipe, Inhibitory action (%) = (1 − As/Ac) ×100, where, As is the absorbance within the sight of the test substance and Ac is the absorbance of control. The IC_{50}: esteem was characterized as the convergence of α-glucosidase inhibitor to restrain half of its action under the measurement conditions.

Antibacterial Activity of MIL and OEL extract (minimal inhibitory concentration MIC)

Antibacterial activity of tested extracts against *H. pylori* was determined by micro-well dilution methods. The inoculum of *H. pylori* was prepared and the suspension to 10^6 CFU/mL. The extracts under investigation and the standard drug (clarithromycin) were prepared in dimethyl sulfoxide (DMSO) and subsequent two-fold dilutions (1000-0.24µg) were performed in a 96- well plate. Each well of the microplate included 40 µl of the growth medium (brain heart infusion (BHI) plus 10 % fetal bovine serum (FBS), 10 µL of inoculum and 50 µl of the diluted extract. The clarithromycin and DMSO are used as a positive and negative control, respectively. The plates were incubated at 37°C for 3 days, in 5% O_2, 10% CO_2, and 85% N_2 atmosphere. After that, 40 µL of 3-(4,5-dimethyl-thiazol-2-yl)-2,5- diphenyl-tetrazolium bromide (MTT) at a final concentration 0.5 mg/ml freshly prepared in water was added to each well and incubated for 30 min. the change to the purple color indicated that bacteria were biologically active. The inhibition percentage was calculated using the given formula:

% inhibition = (Abs Control – Abs Sample) / Abs Control × 100

The concentration of samples (inhibitors) required for 90% of inhibition (MIC_{90}) was determined from corresponding dose-response curves. The MIC was taken to the lowest concentration, where no change of color of MTT was determined using an automatic ELISA microplate reader at 620 nm. The MIC values were done in triplicate.

Statistical Analysis

The outcomes are communicated as means ± SD (Standard Deviation). All tests were performed in triplicate and repeated no less than three times. The measurable contrast between groups was controlled by a one-way analysis of change (ANOVA).

Results

HPLC Analysis of MIL and OEL extracts

Analysis of the MIL and OEL extract by HPLC showed a complex mixture of phenolic and flavonoid compounds (Table 1). Quercitrin was a major compound in MIL (8.61 µg/mg extract), followed by hesperetin and kaempferol (7.87 µg/mg and 6.25 µg/mg, respectively). OEL revealed kaempferol as the main flavonol (9.05 µg/mg extract) followed by quercitrin and hesperetin (8.81 and 7.5 µg/mg extract, respectively). Caffic acid was the major phenolic compound in MIL (3.72 µg/mg), followed by Coumaric acid (3.21 µg/mg), Table 1. Resormarinic acid was the most abundant phenolic compound in OEL extract (3.93 µg/mg).

Table 1: HPLC analysis of Phenolic and Flavonoid components in *Mangifera Indica* (MIL) µg/mg and *Olea europaea* (OEL) µg/mg leaves ethanolic extracts (70% v/v).

		MIL	OEL
		Concentration (µg/mg)	
Phenolic compounds	**Gallic acid**	2.43	3.51
	Resorcinol	1.06	2.49
	Chlorogenic acid	1.37	2.09
	Caffeic acid	3.72	3.62
	Coumaric acid	3.21	2.65
	Ferulic acid	3.06	2.54
	Cinnamic acid	2.44	3.41
	Resormarinic acid	2.68	3.93
	Syringic acid	2.35	3.4
Flavonoid compounds	**Catechin**	4.91	4.75
	Kaempferol	6.25	9.05
	Rutin	3.99	6.78
	Quercetin	8.61	8.81
	Hesperetin	7.87	7.5
	Apigenin	4.77	3.42
	Quercitrin	6.06	7.22

Anti-Inflammatory activity of MIL and OEL

Albumin denaturation.

Denaturation was initiated by increasing the concentrations with bovine albumin under control conditions. Protein denaturation was figured by the assurance of their absorbance. The IC_{50} of OEL extract resulted in 44.02% inhibition of protein denaturation whereas MIL extract showed 44.86% inhibition of protein denaturation when compared with the standard medication 15.12% inhibition of protein denaturation (Table 2).

Histamine release inhibitory (%).

The IC_{50} of MIL extract revealed anti-inflammatory activity more than the olive leave extracts. The percentage of histamine release inhibition was 76.19% for mango leave extract compared to 203.9% for OEL extract and 29.16% for diclofenac (St.) (Table 3).

Table 2: Inhibition of protein denaturation (%) by diclofenac sodium standard (DSS) drug, *Mangifera indica* (MIL) and *Olea europaea* (OEL) leaves ethanolic extracts (70% v/v).

Concentration (µg/mL)	DSS	MIL	OEL
	Inhibition of protein denaturation (%)		
0	0 ±0.00	0 ±0.00	0 ±0.00
7.81	31.12 ± 1.20	22.84 ±1.20	0 ±0.00
15.63	51.21 ±0.58	34.11 ±0.63	6.31 ±1.50
31.25	9.14 ±1.20	46.98 ±1.50	15.42 ±2.10
62.5	68.28 ±0.63	54.37 ±2.10	26.31 ±0.63
125	70.14 ±0.58	57.92 ±1.50	32.18 ±1.50
250	76.52 ±0.63	64.32 ±0.72	44.82 ±0.72
500	84.12 ±1.20	75.38 ±0.58	51.34 ±1.50
1000	89.35 ±0.58	84.35 ±1.50	56.38 ±2.10
***IC_{50}**	15.12	448.6	44.02

Table 3 Anti-inflammatory activity (Histamine release inhibitory %) of Diclofenac (DSS), *Mangifera indica* (MIL) and *Olea europaea* (OEL) leave ethanolic extracts (70% v/v).

Concentration (µg/mL)	DSS	MIL	OEL
	Inhibition of protein denaturation (%)		
0	0 ±0.00	0 ±0.00	0 ±0.00
7.81	36.32 ± 1.50	18.73 ±1.50	27.34 ±0.00
15.63	49.25 ±2.10	21.32 ±1.80	38.14 ±0.58
31.25	54.32 ±0.58	38.97 ±0.72	51.84 ±1.2
62.5	57.32 ±0.63	48.37 ±0.63	59.32 ±1.60
125	61.35 ±2.10	55.81 ±1.50	61.33 ±0.63
250	68.32 ±1.20	59.71 ±2.10	68.37 ±1.50
500	79.25 ±2.50	68.34 ±0.58	74.94 ±1.20
1000	86.34 ±1.50	77.32 ±1.50	79.32 ±0.72
***IC_{50}**	29.16	76.19	203.9

Anti-diabetic activity of MIL and OEL extracts

MIL and OEL extracts (ethanol 70%) showed a remarkable inhibition activity on yeast α-glucosidases. The IC_{50} values indicated that MIL extract showed effects on α-glucosidases inhibition (71.6%) similar to that of acarbose (30.57%), a well-studied strong α-glucosidase inhibitor). Data also showing that OEL extract showed even more strong inhibition influences (129.0%) (Table 4).

Table 4: Anti-diabetic activity (Alpha-glucosidase inhibitory %) of Acarbose (A), *Mangifera Indica* MIL) and *Olea europaea* (OEL) leaves ethanolic extracts (70% v/v).

Concentration (µg/mL)	A	MIL	OEL
	Mean of Alpha-glucosidase inhibitory%		
0	0 ±0.00	0 ±0.00	0 ±0.00
7.81	32.15 ±0.58	14.32 ±2.10	8.32 ±1.20
15.63	43.28 ±1.20	26.84 ±1.80	14.43 ±0.72
31.25	50.31 ±1.50	37.71 ±1.50	21.98 ±0.58
62.5	60.14 ±0.72	49.32 ±0.63	30.81 ±1.50
125	63.42 ±2.10	53.99 ±2.10	49.82 ±1.20
250	71.34 ±1.50	56.28 ±0.72	55.31 ±2.50
500	86.34 ±1.20	66.82 ±0.63	60.35 ±2.10
1000	90.10 ±0.58	71.12 ±0.58	62.34 ±1.30
***IC_{50}**	30.57	71.60	129.00

Anti- Helicobacter pylori activity of MIL and OEL extracts

Treatment for H. pylori can take both common and a customary way. H. pylori normal treatment can't just help annihilate H. pylori contamination yet additionally diminish inflammation all through the gastrointestinal tract and help to decrease the danger of various gut malignant growths. The antibacterial activities of clarithromycin, MIL and OEL extract against *H. pylori*, using spectrophotometer micro dilution assay are shown in Table (5). The results demonstrated that ethanolic 70% extracts exhibited anti-*H. Pylori* activity with MIC value of 0.7, 92.61and 6.6 µg/mL for clarithromycin, MIL and OEL extract, respectively. It is clear from the results that OEL extract provided a significant antibacterial effect against *H. pylori* (Antihelicobacter pylori) more than MIL extract.

Table 5: Anti-*Helicobacter pylori* activity (% *Helicobacter pylori* inhibitory) of clarithromycin (C), *Mangifera indica* (MIL) and *Olea europaea* (OEL) leave ethanolic extracts (70% v/v).

Concentration (µg/mL)	C	MIL	OEL
	***Helicobacter pylori* inhibitory (%)**		
0.00	0 ±0.00	0 ±0.00	0 ±0.00
0.24	81.35 ±1.50	0 ±0.00	12.34 ±2.10
0.48	87.65 ±0.58	0 ±0.00	29.71 ±0.63
0.98	92.45 ±1.20	9.32 ±2.50	57.31 ±2.50
1.95	100 ±0.00	16.32 ±1.50	68.35 ±1.50
3.90	100 ±0.00	33.84 ±0.58	84.37 ±1.50
7.81	100 ±0.00	33.84 ±0.63	92.37 ±0.58
15.63	100 ±0.00	51.37 ±2.10	100 ±0.00
31.25	100 ±0.00	84.31±1.50	100 ±0.00
62.50	100 ±0.00	86.42 ±1.20	100 ±0.00
125	100 ±0.00	93.85 ±0.72	100 ±0.00
***MIC$_{90}$**	0.7	92.61	6.6

Discussion

The HPLC data is showing that OEL extract contains a higher amount of polyphenols than mango leave extract (Table 1). Resormarinic acid was the most abundant phenolic compound found in OEL (3.93 µg/mg extract), and Kaempferol was the major flavonoid compound presented in OEL (9.05 µg/mg extract) compared with MIL. (Caponio *et al.,* 2001) reported that the olive leaves extract was rich in bioactive phenolic compounds. The *in vitro* anti-inflammatory, anti-diabetic and *H. pyroli* inhibition activities of the plants can be attributed to its constituents.

MIL extract contained some phenolic compounds involved as antioxidants, contributing to decreasing in the danger of cardiovascular diseases, while phenolic compounds such as gallic acid and quercetin were found to show activity against allergies, inflammation, hypertension, arthritis, and carcinogenesis (Fernández-Pachón *et al.,* 2004). Rosmarinic acid, a dimer-type caffeic acid subordinate, has demonstrated numerous pharmacological activities, including anti-inflammatory, antimutagenic, cytoprotective, and immunomodulatory impacts (Amoah et al., 2016). Besides numerous mixes were recognized from Croton cajucara, for example, O-glycosides of kaempferol and quercetin, flavonoid-C-glycosylated, tannins and cinnamic acid subordinates. These mixes fractionated by extremity and measured for their calming action, utilizing a model of mice edema, actuated by an intraplantar infusion of carrageenan. All divisions showed exhibited anti-inflammatory activity (Nascimento *et al.,* 2017).

Mango leaves ethanol 70 % extract to give better anti-diabetic activities. The α-glucosidase is available in the brush fringe of the small digestive system. This compound is valuable in catalyzing the breakdown of oligo or disaccharides into basic sugars and in this manner, inhibitors of this chemical can keep down the take-up of dietary starches and in this manner can control the blood glucose level (Hossain *et al.,* 2008). Consequently, the proficiency of ethanol 70 % mango leaves remove in controlling this chemical was assessed (Table 4).

OEL extract provided a significant antibacterial effect against *H. pylori* (Antihelicobacter pylori) more than MLE (Table 5) this concurred with (Eman *et al.,* 2017) they reported that (OLE) had a strong antibacterial effect against the virulent *H.* pylori. Furthermore, (Lee and Lee, 2010) reported that a mixture of phenolic compounds of olive leaves extract has greater antimicrobial activity than those phenolic compounds that were tested individually, the antimicrobial effect of olive leaves is due to its phenolic composition. Two noteworthy monomer type caffeic acid subsidiaries have demonstrated antimicrobial action against an extensive variety of plant pathogens (Zhao *et al.,* 2011).

Conclusion

In conclusion, MIL extract gives better antihistamine and anti-diabetic activities than OEL extract which might be due to its phenolic components. On the other hand, OEL extract showed a good potential therapeutic effect for prevention of albumin denaturation and *H. pylori* activities than MIL extract. These results suggest increasing the use of these materials as dietary supplements or the involvement of these ingredients into food products to increase its beneficial outcomes and improve human health.

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Conflict of interest

The author declares that he has no conflict of interest.

Author Contribution

The author performed the experimental work and the writing of the manuscript.

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