

Scientifically Proven Health Benefits of Oil No. 10

1. Introduction

"Laurus nobilis" in Latin is also known to a layman as Bay leaf, which has been known as a spice and herb for time long. Originating from the Indian subcontinent it is said that it spread in Europe and not only has it entered the cook books, but also even as home remedy for some diseases. In the ancient system of Ayurveda medicine, the bay leaf has been used for many purposes thus suggested as a home remedy. The best way to use this herb is in the form of the leaves itself either in cooking or just plain powder of the leaves. In recent years one can use oil base too too. Recent studies have found have agreed to the basic use of this leaves as astringent, anti bacterial, anti fungal, diaphoretic, diuretic, emetic and stomachic properties.

2. Chemical components of Bay Leaf Oil

Gas chromatography/mass spectrometry analysis of Laurus nobilis essential oil composition of northern Cyprus

The chemical composition of the essential oil isolated from the leaves of the Laurus nobilis plant (from the Northern Cyprus Mountains) by hydrodistillation was analyzed by gas chromatography-mass spectrometry. Of the 81 compounds representing 98.74% of total oil, monocyclic monoterpenes such as 1,8-cineole (58.59%), alpha-terpinyl acetate (8.82%), and

terpinene-4-ol (4.25%) were the main components. Bicyclic monoterpenes such as alpha- and beta- pinene (3.39-3.25%) and sabinene (3.32%) were also identified. The acyclic monoterpenes linalool (0.19%) and myrcenol (0.10%) were present in smaller amounts, and so were the sesquiterpenes. o-Cymene (1.30%) and p-cymene (1.83%) were the main, while cumin aldehyde (0.24%), dimethylstyrene (0.08%), eugenol (0.16%), methyl eugenol (0.05%), and carvacrol (0.05%) were found as minor, aromatic compounds of laurel oil.

Phytochemical composition and antioxidant activity of Laurus nobilis L. leaf infusion

Laurus nobilis L. (laurel) leaves are frequently used as a spice for cooking purposes. Folk medicine in many countries uses the infusion of the plant in stomachic and carminative remedies, as well as for the treatment of gastric diseases. Little information is available about the phytochemical composition of the infusion of dried leaves, which is a way to consume this aromatic and medicinal plant. Phytochemical investigations on the infusion were performed by high-performance liquid chromatography (HPLC) with a diode array detector (DAD) and direct electrospray ionization-tandem mass spectrometry. Several flavonoid derivatives were detected. Semipreparative HPLC from the infusion of laurel leaves isolated 10 flavonoid O-glycosides, one flavonoid C-glycoside, catechin, and cinnamtannin B1. Structures of the isolated compounds were computed on the basis of spectral measurements including high-resolution mass spectrometry spectroscopy and one- and two-dimensional nuclear magnetic resonance techniques. The amount of the flavonoids was also determined by HPLC-DAD. The antioxidant activity of the tea and the isolated compounds was also measured using two different in vitro methods: the Briggs-Rauscher oscillating reaction test, at a pH similar to that of the gastric juice, and the Trolox equivalent antioxidant capacity assay, at the pH of blood. For the infusion and the methanol extract the total phenolic content was also measured using the Folin-Ciocalteu reagent.

Essential oil composition and variability of Laurus nobilis L. growing in Tunisia, comparison and chemometric investigation of different plant organs

Stems, leaves, buds and flowers of *Laurus nobilis* L. growing wild in Tunisia were analysed for their essential oil composition. The essential oil of *Laurus nobilis* L. gathered from different stations were isolated by hydrodistillation and analysed by GC/MS. The oil yields on a dry weight basis ranged between 0.4% and 1.1%. The major component identified was 1,8-cineole, other predominant components were alpha-terpinyl acetate, methyl eugenol, eugenol and linalool. Although the same compounds were present in all plant organs, the leaves differed from the stems in the concentration of 1,8-cineole and methyl eugenol, buds and flowers in the concentration of 1,8-cineole and the stem's oil composition differs from the others in content of methyl eugenol. The results obtained from GC/MS analysis of the volatile oils from individual plant organs were submitted to principal component analysis. Chemometric investigations led to differentiation of stems, leaves and buds-flowers with the respect to the content of 1,8-cineole, methyl eugenol and alpha-terpinyl acetate; flowers and buds were non-differentiated. Finally, the antibacterial activity of the leaves' essential oils has been assayed.

3. Clinical studies

Antimicrobial activities

Bactericidal activities of plant essential oils and some of their isolated constituents against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, and Salmonella enterica

An improved method of sample preparation was used in a microplate assay to evaluate the bactericidal activity levels of 96 essential oils and 23 oil compounds against *Campylobacter*

jejuni, Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella enterica obtained from food and clinical sources. Bactericidal activity (BA50) was defined as the percentage of the sample in the assay mixture that resulted in a 50% decrease in CFU relative to a buffer control. Twenty-seven oils and 12 compounds were active against all four species of bacteria. The oils that were most active against C. jejuni (with BA50 values ranging from 0.003 to 0.009) were marigold, ginger root, jasmine, patchouli, gardenia, cedarwood, carrot seed, celery seed, mugwort, spikenard, and orange bitter oils; those that were most active against E. coli (with BA50 values ranging from 0.046 to 0.14) were oregano, thyme, cinnamon, palmarosa, bay leaf, clove bud, lemon grass, and allspice oils; those that were most active against L monocytogenes (with BA50 values ranging from 0.057 to 0.092) were gardenia, cedarwood, bay leaf, clove bud, oregano, cinnamon, allspice, thyme, and patchouli oils; and those that were most active against S. enterica (with BA50 values ranging from 0.045 to 0.14) were thyme, oregano, cinnamon, clove bud, allspice, bay leaf, palmarosa, and marjoram oils. The oil compounds that were most active against C. jejuni (with BA50 values ranging from 0.003 to 0.034) were cinnamaldehyde, estragole, carvacrol, benzaldehyde, citral, thymol, eugenol, perillaldehyde, carvone R, and geranyl acetate; those that were most active against E. coli (with BA50 values ranging from 0.057 to 0.28) were carvacrol, cinnamaldehyde, thymol, eugenol, salicylaldehyde, geraniol, isoeugenol, citral, perillaldehyde, and estragole; those that were most active against L monocytogenes (with BA50 values ranging from 0.019 to 0.43) were cinnamaldehyde, eugenol, thymol, carvacrol, citral, geraniol, perillaldehyde, carvone S, estragole, and salicylaldehyde; and those that were most active against S. enterica (with BA50 values ranging from 0.034 to 0.21) were thymol, cinnamaldehyde, carvacrol, eugenol, salicylaldehyde, geraniol, isoeugenol, terpineol, perillaldehyde, and estragole. The possible significance of these results with regard to food microbiology is discussed.

Microbial growth and quorum sensing antagonist activities of herbal plants extracts

Antimicrobial and anti-quorum sensing (AQS) activities of fourteen ethanolic extracts of different parts of eight plants were screened against four Gram-positive, five Gram-negative

bacteria and four fungi. Depending on the plant part extract used and the test microorganism, variable activities were recorded at 3 mg per disc. Among the Grampositive bacteria tested, for example, activities of Laurus nobilis bark extract ranged between a 9.5 mm inhibition zone against Bacillus subtilis up to a 25 mm one against methicillin resistant Staphylococcus aureus. Staphylococcus aureus and Aspergillus fumigatus were the most susceptible among bacteria and fungi tested towards other plant parts. Of interest is the tangible antifungal activity of a Tecoma capensis flower extract, which is reported for the first time. However, minimum inhibitory concentrations (MIC's) for both bacteria and fungi were relatively high (0.5-3.0 mg). As for antiquorum sensing activity against Chromobacterium violaceum, superior activity (>17 mm QS inhibition) was associated with Sonchus oleraceus and Laurus nobilis extracts and weak to good activity (8-17 mm) was recorded for other plants. In conclusion, results indicate the potential of these plant extracts in treating microbial infections through cell growth inhibition or quorum sensing antagonism, which is reported for the first time, thus validating their medicinal use.

Antioxidant, Anti-inflammatory and Analgesic effects

The in vitro screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal

Essential oil, ethanolic extract and decoction of 10 plant species from interior Portugal were analyzed for their activity towards acetylcholinesterase (AChE) enzyme and their antioxidant activity. Of these, Melissa officinalis, Paronychia argentea, Sanguisorba minor, Hypericum undulatum and Malva silvestris are used in herbal medicine, Laurus nobilis and Mentha suaveolens as condiments, and Salvia officinalis, Lavandula angustifolia and Lavandula pedunculata also as aromatics. Melissa officinalis and Mentha suaveolens showed AChE inhibitory capacity higher than 50% in the essential oil fraction. Laurus nobilis, Hypericum undulatum, and Sanguisorba minor showed a high inhibition value of AChE in the ethanolic

fraction, 64% (1 mg ml⁻¹), 68% (0.5 mg ml⁻¹), and 78% (1 mg ml⁻¹), respectively. Higher values of AChE inhibitory activity were found using decoctions of *Lavandula pedunculata*, *Mentha suaveolens* and *Hypericum undulatum*, 68, 69 and 82% (at a concentration of 5mg dry plant ml⁻¹ of assay), respectively. The free radical scavenger activity was higher for the polar extracts. In the water extracts most of the plants showed values around 90%. When antioxidant activity was measured with the beta-carotene-linoleic acid assay high activity (65-95%) was also found in the water extracts. *Hypericum undulatum*, *Melissa officinalis* and *Laurus nobilis* showed both high AChE inhibitory capacity and antioxidant activity.

Analgesic and anti-inflammatory activity of the leaf essential oil of Laurus nobilis Linn.

The leaf essential oil of *Laurus nobilis* Linn. (Lauraceae) has been evaluated for antinociceptive and anti-inflammatory activities in mice and rats. The essential oil exhibited: (1) a significant analgesic effect in tail-flick and formalin tests; (2) a dose-dependent anti-inflammatory effect in the formalin-induced edema and (3) a moderate sedative effect at the anti-inflammatory doses. The analgesic and anti-inflammatory effect of the essential oil was comparable to reference analgesics and non-steroid anti-inflammatory drugs: morphine and piroxicam. Present results make the essential oil worthy of further investigations.

Evaluation of in vitro antioxidant activity of Indian bay leaf, Cinnamomum tamala (Buch. -Ham.) T. Nees & Eberm using rat brain synaptosomes as model system

The study investigated the perturbation of oxidant-antioxidant balance in brain synaptosomes of diabetic rats and determined the antioxidant and free radical-scavenging property of the Indian bay leaf. Brain synaptosomes were isolated from control and streptozotocin-induced diabetic animals and oxidative stress parameters were assayed. A methanolic extract of bay leaf (BLE) was tested for the polyphenolic content and antioxidant activity by in vitro assays. A significant increase in the levels of lipids and lipid peroxidation

products and a decline in antioxidant potential were observed in diabetic rat brain synaptosomes. The total polyphenolic content of BLE was found to be 6.7 mg gallic acid equivalents (GAE)/100g. BLE displayed scavenging activity against superoxide and hydroxyl radicals in a concentration-dependent manner. Further, BLE showed inhibition of Fe(2+)-ascorbate induced lipid peroxidation in both control and diabetic rat brain synaptosomes. Maximum inhibition of lipid peroxidation, radical scavenging action and reducing power of BLE were observed at a concentration of 220 microg GAE. These effects of BLE in vitro were comparable with that of butylated hydroxyl toluene (BHT), a synthetic antioxidant. It can be concluded that synaptosomes from diabetic rats are susceptible to oxidative damage and the positive effects of bay leaf in vitro, could be attributed to the presence of antioxidant phytochemicals.

Cinnamtannin B-1 from bay wood reduces abnormal intracellular Ca²⁺ homeostasis and platelet hyperaggregability in type 2 diabetes mellitus patients

Type 2 diabetes mellitus induces a number of cardiovascular disorders, including platelet hyperactivity and hyperaggregability, which is associated to an increased oxidant production and abnormal cytosolic Ca²⁺ mobilization. In the present study, the effect of cinnamtannin B-1 obtained from bay wood on oxidants production, Ca²⁺ mobilization and aggregation in platelets from type 2 diabetic donors is investigated. Pretreatment of platelets with cinnamtannin B-1 reversed the enhanced oxidants production and Ca²⁺ mobilization, including Ca²⁺ entry, evoked by thapsigargin plus ionomycin or thrombin, observed in platelets from diabetic subjects, so that in the presence of cinnamtannin B-1 Ca²⁺ entry was similar in platelets from healthy and diabetic subjects. In addition, cinnamtannin B-1 reduced thrombin-induced aggregation in platelets from type 2 diabetic subjects. It is concluded that cinnamtannin B-1 exerts an effective antioxidant action in platelets from patients with type 2 diabetes mellitus and reverses the enhanced Ca²⁺ mobilization and hyperaggregability.

Inhibitory effects of sesquiterpenes from bay leaf on nitric oxide production in lipopolysaccharide-activated macrophages: structure requirement and role of heat shock protein induction

The methanolic extract from the leaves of *Laurus nobilis* (bay leaf, laurel) was found to inhibit nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages. Through bioassay-guided separation, fourteen known sesquiterpenes were isolated from the active fraction and were examined for ability to inhibit the NO production. Seven sesquiterpene lactones (costunolide, dehydrocostus lactone, eremanthine, zaluzanin C, magnolialide, santamarine and spirafolide) potently inhibited LPS-induced NO production ($IC_{50} = 1.2$ approximately 3.8 μM). Other sesquiterpene constituents also showed the inhibitory activity ($IC_{50} > \text{or} = 21$ μM), but their inhibitory activities were less than those of sesquiterpene lactones. Alpha-methylene-gamma-butyrolactone also showed inhibitory activity ($IC_{50} = 9.6$ μM), while mokko lactone and watsonol A etc., reductants of the alpha-methylene-gamma-butyrolactone moiety by NaBH_4 or DIBAL, and a 2-mercaptoethanol adduct of dehydrocostus lactone showed little activity ($IC_{50} > \text{or} = 18$ μM). These results indicated that the alpha-methylene-gamma-butyrolactone moiety is important for the activity. Furthermore, costunolide and dehydrocostus lactone inhibited inducible nitric oxide synthase (iNOS) induction in accordance with induction of heat shock protein 72 (HSP 72). These results suggested that, as one of their mechanisms of action, sesquiterpene lactones induce HSP 72 thereby preventing nuclear factor-kappaB activation followed by iNOS induction.

Cytotoxic activity against tumor cells

Cytotoxic activity of essential oils from labiatae and lauraceae families against in vitro human tumor models

The aim of this work was to study the cytotoxicity of essential oils and their identified constituents from *Sideritis perfoliata*, *Satureia thymbra*, *Salvia officinalis*, *Laurus nobilis* and *Pistacia palestina*. Essential oils were obtained by hydrodistillation and were analysed by gas chromatography (GC) and GC/mass spectrometry (MS). The cytotoxic activity was evaluated in amelanotic melanoma C32, renal cell adenocarcinoma ACHN, hormone-dependent prostate carcinoma LNCaP, and MCF-7 breast cancer cell lines by the sulforhodamine B (SRB) assay. *L. nobilis* fruit oil exerted the highest activity with IC50 values on C32 and ACHN of 75.45 and 78.24 microg/ml, respectively. The activity of *S. perfoliata* oil on both cell lines (IC50 of 100.90 mg/ml for C32 and 98.58 microg/ml for ACHN, respectively) was also interesting. Among the tested constituents the highest activity was found when α -humulene was applied to LNCaP cells (IC50 of 11.24 microg/ml). This study suggests for the first time the ability of *S. perfoliata*, *S. thymbra*, *S. officinalis*, *L. nobilis* and *P. palestina* essential oils and some identified terpenes to inhibit human tumor cell growth.

Screening of indigenous Palestinian medicinal plants for potential anti-inflammatory and cytotoxic activity

Organic extracts of 24 selected plant species, used by Palestinian traditional healers to treat different illnesses and diseases, were tested for their anti-inflammatory and anti-tumoral activities. The plant selection was based on existing ethnobotanic information and interviews with local healers. The extracts of the plants under investigation were tested for their potential anti-tumor (cytotoxic) effect on the murine fibrosarcoma L929sA cells, and on the human breast cancer cells MDA-MB231 and MCF7. Cytotoxicity screening models provide important preliminary data to select plant extracts with potential antineoplastic properties. MTT (Tetrazolium blue) colorimetric assay was used to evaluate the reduction of viability of cell cultures in the presence or absence of the extracts. The extract from *Withania somnifera*, L. Dunal (Solanaceae) presented an IC(50) value at 24h of 150 and 60 microg/ml, on L929sA and MCF7 cells, respectively, while the extract from *Psidium guajava* L. (Myrtaceae) presented an IC(50) value at 24h of 55 microg/ml on MCF7 cells. Other extracts examined, like *Laurus nobilis* L. (Lauraceae) and *Salvia fruticosa* M. (Labiatae), also displayed a remarkable activity. Additionally, as the nuclear transcription factor NFkappaB

regulates the expression of various genes that play critical roles in apoptosis and immunomodulation, we further investigated the effect of nine promising plant extracts, withheld from the first cell viability screening on NFkappaB activation. The extracts showed variable degrees of NFkappaB-inhibitory activity. Whereas *Withania somnifera* extract demonstrated the strongest NFkappaB-inhibitory activity, other extracts derived from *Laurus nobilis*, *Psidium guajava* and *Foeniculum vulgare* M. (Umbiliferae) also revealed immunomodulatory NFkappaB activities. These species are good candidates for further activity-monitored fractionation to identify active constituents.

Hot water soluble sesquiterpenes [anhydroperoxy-costunolide and 3-oxoeudesma-1,4(15),11(13)triene-12,6alpha-olide] isolated from laurel (Laurus nobilis L.) induce cell death and morphological change indicative of apoptotic chromatin condensation in leukemia cells

Hot water soluble (HWS)-sesquiterpenes [anhydroperoxycostunolide and 3-oxo-eudesma-1,4(15),11(13)triene-12,6alpha-olide] were purified from Laurel (*Laurus nobilis* L.) and identified by Mass, and ¹H- and ¹³C-NMR. These HWS-sesquiterpenes displayed strong growth inhibitory effect against human promyelotic leukemia HL-60 cells. Apoptotic morphological changes of the nucleus, including chromatin condensation were induced in the HL-60 cells treated with the sesquiterpenes. Flow cytometric analysis showed that the hypodiploid nuclei of HL-60 cells were increased to 10.5, 46.5, and 91.3% after a 3 day-treatment with 2.5, 5 and 10 micromoles anhydroperoxycostunolide, respectively. And the same analysis showed that the hypodiploid nuclei of HL-60 cells were increased to 9.8, 39.2 and 89.6% after a 3 day-treatment with 5, 10 and 20 micromoles 3-oxo-eudesma-1,4(15),11(13)triene-12,6alpha-olide, respectively. These findings suggest that growth inhibition by anhydroperoxycostunolide and 3-oxo-eudesma-1,4(15),11(13)triene-12,6alpha-olide of HL-60 cells results from the induction of chromatin condensation in the HL-60 cells. On the other hand, fragmentations by these compounds of DNA to oligonucleosomal-sized fragments were not observed in the HL-60 cells.

Anticonvulsant activity

Anticonvulsant activity of the leaf essential oil of Laurus nobilis against pentylenetetrazole- and maximal electroshock-induced seizures

The leaf essential oil of *Laurus nobilis* Linn., Lauraceae, which has been used as an antiepileptic remedy in Iranian traditional medicine, was evaluated for anticonvulsant activity against experimental seizures. The essential oil protected mice against tonic convulsions induced by maximal electroshock and especially by pentylenetetrazole. Components responsible for this effect may be methyleugenol, eugenol and pinene present in the essential oil. At anticonvulsant doses, the essential oil produced sedation and motor impairment. This effect seems to be related in part to cineol, eugenol and methyleugenol. Although the essential oil had an acceptable acute toxicity, further studies are required before any absolute conclusions can be drawn.

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