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Mangifera indica peels: A common waste product with impressive immunostimulant, anticancer and antimicrobial potency

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Abstract: The volatile components of 3 mango cultivars were investigated by means of GC, and GC-MS. Two hundred and forty compounds were identified, of which eighty five compounds were found for the first time in mango fruit. Terpene hydrocarbons were the major volatiles of all cultivars, the dominant terpenes in the 3 mango cultivars were δ-3-carene (2.784-14.904%), α-terpinolene (4.825-15.879%), α-copaene (2.281-8.097%), and caryophyIlene (3.390-10.897%). peel essential oils of *M. indica* cultivar zebdeya and *M. indica* cultivar cobaneya showed appreciable non specific immunostimulant activity measured by low Macrophage migration index. *M. indica* cultivar zebdeya and *M. indica* cultivar cobaneya had higher phagocytic indices up to 1.47and 1.06 respectively. The cytotoxicity of the essential oils were studied *invitro* against MCF-7, HCT-116 and Hep-G2 cancer cell lines using MTT assay showed significant effect with IC_{50} = 1.62-1.77, 2.95-5.56 and 2.76-3.14 µl/ml for respectively. Interestingly, *M. indica* cultivars zebdeya, cobaneya and hindi expressed a valuable cytotoxic effect on MCF-7 breast carcinoma cell line as compared to standard antitumor drug.

The essential oils *M. indica* cultivars were tested by Kirby-Bauer method showing a wide range of antibacterial and antifungal activities. Generally, essential oils of the *M. indica* cultivars showed a moderate to high antibacterial activity with *M. indica* cultivar hindi being the most active showing broad spectrum activity against Gram-positive (18-21 mm) and Gram-negative bacteria (16-19 mm). It also showed significant antifungal activity against *Candida albicans* (16 mm); however, data revealed that all *tested cultivars* were ineffective and have no antifungal activity against *Aspergillus flavus.*

The MIC of the three *M. indica* cultivars essential oils revealed that although essential oils didn't show antimicrobial activities against *Aspergillus flavus* the peel essential oils demonstrated variability in the inhibitory concentrations of each essential oil for the given microorganisms. The essential oils of peels showed activities in the range (concentrations) from 116 to 142 µl/ml for *M. indica* cultivar zebdeya, 58 to 89 µl/ml for *M. indica* cultivar hindi and from 121 to 172 µl/ml for *M. indica* cultivar cobaneya. The present investigation proved the possibility of using *M. indica* peel waste as a source of low-cost natural immunostimulant, anticancer and antimicrobial.

Key words: *Mangifera indica* peels; waste product; immunostimulant, anticancer; antimicrobial

1. Introduction

In view of the beginning of human civilization, we have been exploiting our natural resources for eradication of common human pathogen borne diseases. Various developing countries over the globe have been ravaged by bacterial pathogen borne diseases. Mostly, three diseases, namely, tuberculosis, malaria, and AIDS attributes to the majority of infectious diseases. These diseases on a whole, proves fatal for the human population of 5 million and causes sickness to the human population of 300 million each year, all over the globe (WHO, 2002). These infectious diseases can be prevented by controlling the growth of food borne pathogenic microorganisms and food spoilage.

Mango (*Mangifera indica* L.), a fruit belongs to the family Anacardiaceae, which comprises about 70 genera. Historical records suggest that its cultivation as a fruit tree originated in India more than 4000 years ago (Mukherjee, 1997). With a growing world production, the mango represents one of the most important tropical fruits and is produced worldwide. It is mostly found in tropical countries like India. Various products are found in India as mango processed food products. Mango is mostly used in food processing industries such as Juice industries, jam industries, jelly industries, and pickle industries These processed food leads to enormous generation of mango peel as a waste product. It needs a huge capital to decompose these peels to make sure that it does not pollute the environment. To save this investment in the disposal of mango peels, it can be converted as a raw material for pharmaceutical industries based on the result of the current research work. These waste products can be utilized for their immunostimulant, anticancer as well as antimicrobial activities. Hence, the utilization of mango by-products especially mango peels may be an economical way to reduce the problem of waste disposal from mango production (Kittiphoom, 2012).

Numerous scientific investigations point at consecutive rich eco-friendly sources of immunostimulant, anticancer and antimicrobial properties, especially among fruits and vegetables, but only few of them involve waste parts of fruits, i.e. seeds and peels. Many of the fruits and vegetables skins are thrown in the garbage or fed to livestock. Fruits and vegetables wastes and by-products, which are formed in great amounts during industrial processing, represent a serious problem, as they exert an influence on environment and need to be managed and/or utilized. On the other hand, they are very rich in bioactive components, which are considered to have a beneficial effect on health. Since last decade, efforts have been made to improve methods and ways of reusing fruits and vegetables wastes. The important purpose is the valorization of the biocomponents in byproducts from fruit and vegetable industries. Plant waste is prone to microbial spoilage; therefore drying is necessary before further exploitation. Till now, agro industrial waste often is utilized as feed or fertilizer. But using this agro waste therapeutically is a new idea which is slowly gaining popularity. They are high value products and their recovery will be economically attractive. These are novel, natural, eco friendly and economic sources of antimicrobics, which can be used in the prevention of diseases caused by pathogenic microbes and also reduce pollution.

Immunostimulant compounds are those compounds that enhance body's immune system that may in turn play a major rule in cancer prevention while, antimicrobial compounds are the agents which suppress the growth of bacteria and fungi that can also be fatal. The bacterial pathogens are becoming resistant to the commercially available antibiotics in the market **(**Goosens *et al*., 2005; Mathew *et al*., 2007**)**. This is because of the random use of these drugs. It creates a need to find a new bioactive source of immunostimulant, anticancer and antimicrobial compounds. Thus, the research work was carried on to get an idea of immunostimulant, anticancer and antimicrobial property in mango peels.

Cancer is an abnormal type of tissue growth in which the cells exhibit an uncontrolled division, relatively in an autonomous fashion, leading to a progressive increase in the number of dividing cell (Kanchana and Balakrishna, 2011). There is increasing demands for anticancer therapy (Unno *et al.,* 2005). *Invitro* cytotoxicity testing procedures reduces the use of laboratory animals (Abraham *et al.*, 2004) and hence use of cultured tissues and cells have increased (Byrd *et al.*, 2003).

The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immuno-therapies (Xu *et al.,* 2009). With this aim, many attentions have been paid to natural compounds and essential oils from plants.

Infectious diseases are leading cause of death worldwide. Natural products provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Because of increasing threat of infectious diseases, the need of the hour is to find natural agents with novel mechanism of action. Fruit and vegetable peels are thrown into the environment as agro waste which can be utilized as a source of antimicrobics. It will be economic, eco-friendly and reduce pollution. Here we report the antimicrobial activity of peels essential oils against different microorganisms.

Unlike the immunostimulant, anticancer and antimicrobial agents synthesized by chemical sources, those from natural sources are readily accepted by the consumers. Thus, the development of new immunostimulant, anticancer and antimicrobial agents is a major research opportunity for the researchers at present.

In the, present investigation, we report the immunostimulant, anticancer and antimicrobial properties of peels of three cultivars of *M. indica* fruits that are commonly available and readily consumed in Egypt, and to indicate which of them can become a new source of natural immunostimulant, anticancer and antimicrobial for pharmaceutical industries.

2. Materials and methods

2.1. Plant material:

Fruits of *M. indica* cultivars; namely zebdeya, hindi and cobaneya were collected at the ripening stage. The plants were authenticated by Prof. Dr. Monir Mohamed Abdelghany, The Herbarium, Botany Department, Faculty of Science, Cairo University. Edible parts of the fruits were removed and fruits peels were frozen until used for essential oil preparation.

2.2. Chemicals:

Penicillin, Streptomycin, Amphotericin B, Erythromycin, Gentamycin, (FCS), RPMI 1640, glutamine and HEPS (Sigma, USA), Mueller- Hinton broth (Oxoid, England). All other chemicals and reagents used were of the highest commercially available purity.

2.3. Preparation of essential oils

The fresh mango cultivars peels (1 kg for each cultivar) were cut into small pieces and subjected to hydrodistillation for 5 h, using a Clevenger-type apparatus. Before analysis and biological activity test, the collected oils were dehydrated with anhydrous Na_2SO_4 and preserved at 4°C (Egyptian Pharmacopeia; 2005).

2.4. Gas Chromatography-Mass Spectrometry

The mango peels oils were subjected to gas chromatographic-mass spectral analysis on an Agilent system consisting of a model 6890 gas chromatograph, equipped with a model 5973 mass selective detector (EIMS, electron energy, 70 eV), and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a DB-5 (5% phenyl methyl polysiloxane) stationary phase, film thickness of 0.25 μm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a flow rate of 1.0 ml/min. Inlet temperature was 200°C and MSD detector temperature was 270°C. The mass spectrometer was operated in electron impact ionization (EI) mode with 70eV energy. The mass range was 50-700 Da and the ion source temperature was 200 °C. The GC oven temperature program was used as follows: 80°C initial temperature, for 2 min.; then programmed at 15°C/min to 270°C and held for 10min.Each sample was dissolved in acetone to give a 1% w/v solution; 1 μL injections using a splitless injection technique were used.

Identification of oil components was achieved based on their retention indices (RI, determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Pino *et al*., 2005; Ansari *et al*., 2004; Dzamic *et al*., 2008; Pandit *et al*., 2009; Pino *et al*., 2010) and stored on the MS libraries [NIST 05; Mass Finder database (G1036A, revision D.01.00 and Wiley7 Mass Finder]. The chemical compositions of the essential oils are compiled in Table 1 and Figure 1.

2.5. Immunostimulant activity:

Essential oils of *M. indica* fruit peels under investigation were dissolved in Hank's solution in different concentrations (100, 500, 1000, 1500 and 2000 µl. ml^{-1}). The tested samples were sterilized by filtration through 0.2 µm pore size filters and by addition of 10,000 µg penicillin and 10,000 µg streptomycin antibiotics. Albino mice of either sex (20-22 g) were used for the following:

2.5.1. Preparation of murine (mice) spleen cells:

Spleenocytes were prepared according to conventional procedures, from aseptically removed mouse spleens. The cells were washed three times in RPMI 1640 medium and resuspended in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 10 mM HEPS, pencillin 100 µg ml^{-1}, streptomycin 100 µg ml^{-1} at a final concentration of 3 x 10 cells ml^{-1}. Cell viability was evaluated by trypan blue exclusion test.

2.5.2. Lymphocyte cell culture:

Primary lymphoid cell culture was performed by isolating lymphocytes directly from mice spleens. The lymphocytes then were grown in a chemically defined growth medium RPMI 1640 supplemented with 10% FCS.

2.5.3. Macrophage culture:

Spleen cell suspension was incubated in flat bottomed microtiter plates at 37°C for 7 hours to allow the cells to adhere to the plates then the medium was removed and the adherent cells were washed three times with RPMI 1640. More than 95% adherent cells were macrophage. The cells were cultured with different concentrations of each plant essential oil for 24 hours. The nonspecific immune response of stimulated macrophages and lymphocytes were assayed, by determination of the macrophage migration index and phagocytic index test in which the viable cells were counted using freshly filtered trypan blue stain and haemocytometer slide (Hifnawy *et al.,* 2006).

2.5.4. Macrophage Migration Inhibition Index:

Using a 2 ml syringe filled with silicone, grease the migration chambers (Sterilin) around the rim. Place one dab of grease inside the rim to hold the capillary in place. Prepare the essential oil dilutions in suitable RPMI medium containing 10% fetal calf serum (FCS). Spleen macrophages, of albino mice of either sex (20-22 gm), adherent cells in RPMI medium, washed with the same media, suspended in complete RPMI media containing 10% fetal calf serum (FCS), Resuspend the cells in RPMI medium containing 10% fetal calf serum at a suitable final cell concentration (2×10^6/ml). Suspension was packed into 6 microheamatocrit capillary tubes (Gelman-Hawksley, haematocrit tubes) of uniform diameter (7.5 cm length X l .0-1.2 mm i.d.) by capillarity, one end of the capillary was sealed and plug with wax. Centrifuge at 300 rpm for 5 minutes at room temperature. Score the capillaries at the cell-fluid interface using a diamond pen. Break the capillary at the score-line with forceps. Immediately mount one capillary per migration chamber and fill with RPMI medium containing 10% fetal calf serum, add the plant extract dilution to the well. Cover the migration chamber with a cover-slip ensuring an air-tight seal and no bubbles. Repeat until all the chambers are filled. Incubate the plates on a completely horizontal surface at 37^0C overnight, (15-18 hours). Project the areas of the migrating cells onto plain paper and draw around the outer margin of the migrating fan. Determine the area of migration by planimetry for treated and untreated macrophages. Express the results as the migration inhibition index (MI) determined from:

$$\text{Macrophage Migration Inhibition Index (MI)} = \frac{\text{Area of migration from essential oil treated cells}}{\text{Area of migration from untreated cells (control)}}$$

Macrophage migration index decrease explains the inhibition of migration of macrophages, which in turn causes liberation of cytokines (i.e. immunostimulation). Any essential oil that causes decrease in the macrophage migration index is considered to have immunostimulant activity.

At least three replicate capillaries (1 per chamber) were set up for each test, the test being one dilution of essential oil or culture supernatant or the control (medium and fetal calf serum alone). The results were presented as mean ± standard error (S.E.). Student T test was used for the statistical analysis of data. Results with $p < 0.01$ were considered as statistically significant and were presented in table 2.

2.5.5. Phagocytic Index:

Make up a stock solution of Dow latex particles, 1.0 μm in diameter (Digby Chemical Services) as follows: Suspend 10 μl of latex in 5 ml of Hank′s balanced salt solution or Minimum Essential Medium (MEM). Centrifuge 200-300 g for 10-15 minutes. Collect the supernatant and count the number of particles per ml. Adjust to (5-10 x 10^8/m1) in MEM and store in small aliquots at 4^0C. Pellet the cells under investigation (macrophages) in a round bottomed plastic tube at 1500 rpm for 10 minutes, decant the supernatant. Add 0.2 ml stock latex and 0.2 ml MEM + 20% FCS. Incubate at 37^0C for 1-1.5 hours. Wash 3 times at 200 g, and then add the tested concentration of plant extract and incubate for 24 hr. at 37^0C. Count the number of phagocytosed and non- phagocytosed cells. Divide the number of phagocytosed cells over the non-phagocytosed ones to calculate the treated cells and similarly do that for the control (untreated) cells.

Phagocytic Index = essential oil treated cells / untreated cells.

Phagocytic index increases directly proportional to immunostimulant activity. The results were presented as mean ± standard error (S.E.). Student T test was used for the statistical analysis of data. Results with $p < 0.01$ were considered as statistically significant and were presented in table 3.

Macrophage Migration Inhibition Index (Table 2) and Phagocytic Index (Table 3) were determined relative to the well known immunostimulant drug (*Echinacea purpurea*) root extract as a standard.

2.6. Anticacer activity:

2.6.1. Cancer cell lines:

Three human adenocarcinoma cell lines; breast adenocarcinoma cell line (MCF-7), colon adenocarcinoma cell line (HCT-116) and liver adenocarcinoma cell line (HEP-G2) were obtained from National Institute of Cancer, Cairo University, Cancer biology department, pharmacology unit, Cairo, Egypt. Cells were routinely cultured in DMEM (Dulbecco's Modified Eagle's Medium), which was supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/ml penicillin G sodium, 100 units/ml streptomycin sulphate, and 250 mg/ml Amphotericin B.

2.6.2. Evaluation of invitro cytotoxic activity of the essential oils on tested cell lines:

MTT assay was performed to determine the cytotoxic property of *M. indica* cutivars essential oils against MCF-7, HCT-116 and HEP-G2 cell lines (Van Meerloo *et al*., 2011). Briefly cell lines were seeded in 96-well tissue culture plates. Appropriate concentrations of stock solution (0.5, 5.0, 10.0, 20 µl/ml) were added and incubated for 48 hours at 37^oC. Non-treated cells were used as negative control and doxorubicin as positive control. Incubated cultured cell was then subjected to MTT (3-(4,5 Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) colorimetric assay. The tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) is used to determine cell viability in assays of cell proliferation and cytotoxicity. MTT is reduced in metabolically active cells to yield an insoluble purple formazan product. Cells were harvested from maintenance cultures in the exponential phase and counted by a hemocytometer using trypan blue solution. The cell suspensions were dispensed (100µl) in triplicate into 96-well culture plates at optimized concentrations of 1×10^5/well for each cell lines, after a 24 hours recovery period. Assay plates were read using a spectrophotometer at 520 nm. The spectrophotometrical absorbance of the samples was measured using a microplate (ELISA) reader. The cytotoxicity data was standardized by determining absorbance and calculating the correspondent essential oil concentrations.

Cell viability (%) = Mean OD/ control OD × 100

The IC_{50} values (concentration of essential oil required to kill 50% of cell population i.e. concentration at which 50% of cells were dead) for colon adenocarcinoma (HCT-116), breast adenocarcinoma (MCF-7) and liver carcinoma (Hep-G2) cell lines are reported as mean± S.D. of three independent experiments. IC_{50} values that were greater than 30µl/ml considered insignificant, and vice versa. (Table 4)

2.7. Antimicrobial activity:

Antimicrobial activity of the essential oils and different cultivars of the *M. indica* was screened using Kirby-Bauer disc diffusion method (Bauer *et a1*., 1966) with slight modification. 100 µl of the test bacteria/fungi were grown in 10 ml of fresh media (Mueller-Hinton agar plates (HiMedia) for bacteria and Potato Dextrose Agar plates for fungi (HiMedia)) until they reached a count of approximately 10^8cells/ml for bacteria and 10^5cells/ml for fungi. 100 µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disc diffusion method.

Plates inoculated with filamentous fungi as *Aspergillus flavus* Link (ATCC 204304) at 25°C for 48-72 hours; Gram-positive bacteria as *Staphylococcus aureus* (ATCC 12600) and *Bacillus subtilis* (ATCC 6051); Gram-negative bacteria *Escherichia coli* (ATCC 11775) and *Pseudomonas aeuroginosa* (ATCC 10145) they were incubated at 35-37° C for 24-48 hours and yeast as *Candida albicans* (ATCC 26555) incubated at 30°C for 24-48 hours. Then the diameters of the inhibition zones were measured in millimeters.

DMSO with a concentration up to 2% was used to dissolve the essential oils. Filter paper discs (6 mm in diameter) saturated with 20µL of the tested essential oils and extracts or DMSO (solvent control) were placed on the surface of the inoculated plates. The plates were incubated at 37°C for 24 h. The diameter of the inhibition zone was measured in millimeter with slipping calipers of the National Committee for Clinical Laboratory Standards., and was recorded as mean ± SD of a triplicate experiment. Standard discs of Gentamycin (10µg, Oxoid, UK**,** Antibacterial agent), Amphotericin B (5µg, Sigma Chemical Co., St. Louis, Mo., Antifungal agent) served as positive controls for antimicrobial activity but filter discs impregnated with 10 µl of solvent (DMSO) were used as a negative control. Results were shown in table 5.

Determination of the Minimal Inhibitory Concentration (MIC) was carried out by a serial broth dilution method described by (NCCLS, 1993). Briefly; The essential oils and extracts were diluted in DMSO and were added to 5 ml sterile Muller Hinton Broth tubes to give different concentrations (1.0 - 50.0 µL/ml). Later, 0.5 ml of the exponentially growing microbial broth culture of the strains that were sensitive by disc diffusion test was inoculated into respective test tubes. Another set of tubes containing only the growth medium without DMSO (control) and with DMSO (solvent control up to 2% in the culture media) showed no inhibitions in preliminary studies whereas Gentamicin was used as a positive control for bacterial strains, Amphotericin B was used as a positive control for fungi and each of the test strains was set up separately. In 96-well plates organisms, at a concentration of approximately 1.5 x 10^8 colony forming units (CFU)/ml, were added to each well. The tubes were incubated at 37°C for 24 h and the growth was measured by measuring optical density at 520 nm using spectrophotometer comparing the sample readout with the non inoculated nutrient broth. The MIC was regarded as the lowest concentration (without turbidity) of the essential oil that inhibited the growth of bacteria or fungi. The plates were done in triplicate. Antimicrobial MIC results are listed in table 6.

3. Statistical analysis:

Statistical analysis was performed using SPSS (statistical package of social sciences, version 16). Student T test was used for the statistical analysis of data. Statistical significance was acceptable to a level of $p < 0.01$.

4. Results and discussion:

4.1. Essential oil analysis:

Two hundred and forty compounds were identified, of which eighty five compounds were found for the first time in mango fruit. Terpene hydrocarbons were the major volatiles of all cultivars. The dominant terpenes in the 3 mango cultivars were δ-3-carene (2.784 - 14.904%), α-terpinolene (4.825 - 15.879%), α-copaene (2.281 - 8.097%), and caryophyIlene (3.390 - 10.897%), ethyl tetradecanoate (0.693-1.260 %), 2-heptadecanone (0.895 - 5.144 %), hexadecanoic acid (1.215 - 4.612%) and ethyl hexadecanoate (0.543- 4.029%) that could considered as marker phytoconstituents of mango species. Some components were found only in 2 cultivars in an appreciable amounts as menth-1-ene-4,8-Diol (3.079- 4.570%), α-humulene (2.183 – 5.819 %), caryophyllene oxide (2.897- 4.901 %), tetradecanoic acid (1.168 – 1.384%),1-hexadecanol (4.040 – 10.025 %). Also some components were found in relatively high amounts as p-cymene (13.390%), 1-(1, 1-dimethylethyl)-3-methyl benzene (1.203%), pinan-2-ol (1.081%), menthol (0.450%), cis-calamenene (0.674%), methyl hexdecanoate (0.145%), nonacosane (1.074%) in *M. indica* cultivar Zebdeyia and pentanal (0.111%), 2-methylpropanoic acid (0.890%), cyclohexanol (0.190%), 2,5-hexanedione 0.432%), hexanoic acid (0.230%), 1-(1, 1-dimethylethyl)-4-methyl benzene (0.513%), 1-(2-methylphenyl) ethanone (26.214%), methylchavicol (0.131%), pulegone (0.730%), 2,6,6-trimamethyl-1-cyclohexene-1-acetaldehyde (0.111%), α-terpinene, -7-al (0.890%) and trans-carvyl acetate (0.151%),geranyl acetate (0.121%), α-cedrene (0.211%), 9-epi-(E)-caryophyllene (0.112%), γ-muurolene (0.311%), (E)-β-ionone (0.102%), δ-decalactone (0.190%), pentadecane (0.441%), Tridecanal

Table1. Identified constituents of essential oils of *Mangifera indica* cultivars

No.	Compound	KI	ID	Area %		
				M. Z.	M. H.	M. C.
1	acetaldehyde	528	A	t	0.013	t
2	1-propanol	568	A	0.124	t	--
3	ethyl acetate	605	A	--	0.019	--
4	l-panten-3-ol	673	A	0.029	--	t
5	l-panten-3-one*	678	A	0.102	t	--
6	2,3-pentanedione*	696	A	--	--	0.174
7	pentanal	698	A	--	0.111	--
8	3-pentanone	700	A	0.135	t	--
9	ethyl propanoate	714	A	0.050	--	--
10	methyl methacrylate*	732	A	0.103	t	0.037
11	(E)-3-penten-2-one	735	A	0.203	--	--
12	ethyl 2-methylpropanoate	755	A	0.104	t	0.024
13	3-methyl-2-buten-1-ol	778	A	t	--	0.032
14	2,4-pentanedione	783	A	t	t	--
15	2-methylpropanoic acid*	785	A	t	0.890	--
16	butanoic acid	790	A	--	t	1.032
17	ethyl butanoate	802	A	0.234	0.023	0.035
18	2-methyltetrahydrofuran-3-one	804	A	--	t	--
19	2-furfural	830	A	0.321	0.212	--
20	ethyl 3-methyl butanoate	847	A	0.067	0.890	--
21	(E)-2-hexenal	854	A	0.768	0.098	0.327
22	methyl 3-hydroxy butanoate*	858	A	0.123	t	t
23	1-hexanol	867	A	0.223	0.021	--
24	4-heptanone*	869	A	t	t	--
25	cyclohexanol	886	A	t	0.190	--
26	2-heptanone	889	A	--	t	1.062
27	cyclohexanone	895	A	0.234	0.023	0.034
28	propyl butanoate	896	A	--	t	--
29	heptanal	899	A	0.322	0.212	--
30	santolinatriene*	908	B	0.017	0.890	--
31	butyl propanoate*	910	A	0.468	0.098	0.327
32	tricyclene	923	B	0.132	t	t
33	methyl hexanoate	924	A	0.201	0.045	t
34	α -thujene	931	A	t	--	--
35	2,5-hexanedione*	933	A	--	0.432	--
36	α-pinene	939	A	--	--	5.579
37	2-methylene cyclohexanol*	941	B	0.835	--	--
38	ethyl-3-hydroxy butanoate*	945	A	0.022	0.212	--
39	camphene	953	A	0.037	0.890	--
40	ethyl- 2,3-epoxy butanoate*	962	B	0.498	0.098	0.327
41	5-methyl-2-furfural	964	A	0.176	t	t
42	sabinene	976	B	0.288	0.065	t
43	β-pinene	980	A	t	--	--
44	hexanoic acid	981	A	--	0.230	--
45	β-myrcene	991	A	0.415	--	2.820
46	butyl butanoate	994	A	--	t	0.370
47	2-carene	995	A	--	--	0.121
48	ethyl hexanoate	996	A	0.042	--	--
49	α-phellandrene	1005	A	0.212	0.098	0.211
50	δ-3-carene	1011	A	14.904	2.784	13.583
51	1,4-cineole*	1016	A	0.121	0.011	--
52	α-terpinene	1018	A	--	--	0.216
53	p-cymene	1026	A	13.390	--	--
54	limonene	1031	A	--	t	5.575
55	1,8-cineole	1032	A	t	--	--
56	(E)-β-ocimene	1050	B	--	0.030	--
57	γ -terpinene	1062	A	0.215	--	0.130
58	2,5-dimethyl-4-methoxy-3(2H) furanone	1065	A	--	t	0.070
59	acetophenone	1066	A	--	--	0.121
60	o-tolualdehyde*	1067	A	0.042	--	--
61	β-terpinene	1071	B	--	--	0.741
61	1-(1, 1-dimethylethyl)-3-methyl benzene	1072	B	1.203	--	--
63	dihydromyrecenol	1073	A	t	--	0.011
64	1-(1, 1-dimethylethyl)-4-methyl benzene	1075	C	--	0.513	--

Table 1. Continue

65	p-tolualdehyde	1079	A	--	t	0.211
66	α-terpinolene	1087	A	15.879	4.825	7.940
67	p-cymenene	1089	A	--	t	0.151
68	ethyl heptanoate	1095	A	t	--	0.133
69	linalool	1098	A	t	--	0.098
70	cis-thujone*	1102	A	--	t	--
71	p-1,3,8-menthatriene*	1110	B	0.015	--	1.032
72	α-fenchone	1112	A	0.021	--	
73	isophorone*	1118	A	--	--	0.234
74	cis-p-2-menthen-l-ol	1121	B	--	0.095	--
75	methyl octanoate	1126	A	t	0.049	t
76	ethyl 3-hydroxy hexanoate	1133	A	--	0.087	--
77	pinan-2-ol*	1139	B	1.081	--	--
78	neo-allo-ocimene*	1140	B	--	--	2.267
79	camphor	1143	A	t	0.023	t
80	4-ketoisophorone*	1142	B	0.100	t	--
81	trans-verbenol	1144	B	--	0.019	--
82	isopulegol	1146	A	0.020	t	t
83	menthone	1154	A	0.102	t	--
84	borneol	1164	A	--	--	0.114
85	p-tolyl acetate	1166	A	--	0.111	--
86	p-mentha-1,5-dien-8-ol*	1167	B	0.135	t	--
87	menthol	1173	A	0.450	--	--
88	1-(2-methylphenyl) ethanone*	1176	B	--	26.214	1.630
89	terpinen-4-o1	1177	A	0.236	t	--
90	limonene-4-ol*	1178	B	t	0.013	t
91	methyl phenyl acetate*	1179	A	0.129	t	--
92	octanoic acid	1180	A		0.019	
93	p-cymen-8-ol	1183	B	0.019	--	t
94	butyl hexanoate	1188	A	0.602	t	--
95	α-terpineol	1189	A	--	--	0.134
96	methylchavicol*	1195	A	--	0.131	--
97	ethyl octanoate	1196	A	0.105	t	t
98	trans-dihydrocarvone*	1200	A	0.250	--	--
99	verbenone	1204	A	0.113	t	0.037
100	decanal	1205	A	0.103	t	--
101	α-methylcinnamaldehyde*	1207	A	0.134	t	0.011
102	cis-carveol	1229	A	t	--	0.032
103	Z-3-hexenyl 2-methyl butanoate	1231	B	t	t	--
104	pulegone*	1237	A	t	0.730	--
105	cuminaldehyde	1239	A	--	t	1.032
106	carvone	1242	A	0.234	0.023	0.035
107	eucarvone	1245	B	--	0.019	--
108	trans-2,8-p-menthadiene-1-ol	1246	B	0.019	--	t
109	Isopentyl hexanoate*	1250	A	0.133	t	--
110	p-anisaldehyde*	1251	A	--	--	0.174
111	2,6,6-trimamethyl-1-cyclohexene-1-acetaldehyde*	1254	B	--	0.111	--
112	geraniol	1255	A	0.132	t	--
113	Benzyl propanoate	1257	A	0.150	--	--
114	γ-octalactone	1260	A	--	t	0.037
115	(E)-2-decanal	1261	A	0.103	--	t
116	(E)-cinnamaldehyde*	1266	B	0.100	t	0.024
117	citronellyl formate*	1275	A	t	--	0.042
118	Nonanoic acid	1280	A	--	t	--
119	α-terpinene -7-al*	1282	B	t	0.890	--
120	bornyl acetate*	1285	A	--	t	1.032
121	safrole	1287	A	0.234	0.013	0.025
122	thymol	1290	A	--	0.019	--
123	ethyl nonanoate*	1294	A	0.029	--	t
124	carvaeol	1298	A	0.102	t	--
125	undecanal	1306	A	--	--	0.134
126	trans- carvyl acetate	1337	B	--	0.151	--
127	α-terpinyl acetate	1350	A	0.135	t	--
128	citronellyl acetate	1354	A	t	t	0.032
129	eugenol	1356	A	--	0.095	--

	Table 1. Continue					
130	γ-nonalactone	1360	A	--	t	0.210
131	menth-1-ene-4,8-Diol*	1371	B	3.079	4.570	--
132	α-copaene	1376	B	8.097	2.281	4.757
133	butyl benzoate*	1377	A	t	--	t
134	2-butyl-2-octenal*	1378	B	0.112	--	--
135	decanoic acid	1380	A	0.123	0.042	--
136	geranyl acetate	1383	A	--	0.121	--
137	p-elemene	1391	B	0.122	--	--
138	octyI isobutanoate*	1394	A	0.100	0.012	t
139	ethyl decanoate	1397	A	0.164	0.440	--
140	α -gurjunene	1407	B	--	--	0.054
141	α-cedrene*	1409	B	t	0.211	
142	caryophyIlene	1418	A	6.183	3.390	10.897
143	α -guaiene	1439	B	0.112	0.093	--
144	2-phenylethyl butanoate	1440	A	t	--	0.032
145	benzyl pentanoate*	1445	B	--	0.030	--
146	α -humulene	1454	A	2.183	--	5.819
147	9-epi-(E)-caryophyllene*	1465	B	--	0.112	--
148	γ-decalactone	1470	A	0.116	--	--
149	γ-gurjunene	1472	B	--	t	0.043
150	γ-muurolene	1477	B	t	0.311	--
151	germacrene D	1480	B	--	--	0.798
152	β-selinene	1484	B	0.602	0.828	3.032
153	(E)-β-ionone	1488	A	t	0.102	0.027
154	valencene	1491	A	0.112	t	t
155	α-selinene	1493	B	0.213	0.021	t
156	α –zingiberene*	1495	B	t	t	--
157	δ-decalactone	1490	A	t	0.190	--
158	viridlflorene*	1497	B	--	t	1.052
159	ethyl undecanoate*	1498	A	0.368	0.098	0.327
160	pentadecane*	1500	A	--	0.441	--
161	β-bisabolene*	1509	B	t	--	--
162	tridecanal*	1511	A	--	0.220	--
163	7-epi-α-selinene	1517	B	0.015	--	t
164	β-sesquiphellandrene*	1524	B	--	t	0.170
165	methyl dodecanoate	1526	A	--	--	0.121
166	cis-calamenene	1529	B	0.674	--	--
167	δ- cadinene	1530	B	--	--	0.497
168	cadina-l,4-diene	1532	B	t	0.098	--
169	α-cadinene*	1538	B	t	t	0.077
170	germacrene B*	1556	B	--	--	0.798
171	caryophyllene alcohol*	1564	B	t	0.032	--
172	(E)-nerolidol	1568	A	t	--	--
173	γ-undecalactone	1573	A	--	0.030	--
174	dodecanoic acid	1580	A	0.015	--	0.120
175	caryophyllene oxide	1581	A	2.897	4.901	--
176	2-phenylethyl tiglate*	1584	A	t	0.049	t
177	butyl decanoate	1588	B	--	0.017	--
178	humulene epoxide	1593	B	0.081	--	--
179	ethyl dodecanoate	1597	A	--	--	2.374
180	humulene epoxide II	1606	B	--	--	5.579
181	tetradecanal	1611	A	0.535	--	--
182	γ-eudesmol*	1629	B	t	0.212	--
183	methyl tridecanoate*	1631	A	t	0.021	--
184	τ-muurolol*	1641	B	0.094	0.065	0.327
185	cubenol*	1642	B	0.026	t	t
186	α-muurolol*	1643	B	--	0.548	--
187	selin-11-en-4-α-ol	1652	B	t	0.221	0.213
188	α-cadinol	1654	B	0.276	0.434	--
189	ar-turmerone*	1664	B	--	--	5.579
190	bulnesol	1666	B	0.335	--	--
191	(Z)-3-hexenyl salicylate*	1670	A	0.012	0.212	--
192	cadalene*	1673	B	t	0.290	--
193	γ-dodecalactone	1675	A	0.198	0.098	0.327
194	tridecanoic acid*	1678	A	0.106	t	t
195	propyl dodecanoate*	1685	B	0.108	0.025	t

	Table 1. Continue					
196	(Z)-11-pentadecenal *	1686	B	t	--	--
197	ethyl tridecanoate*	1687	A	--	0.230	--
198	2-pentadecanone*	1697	A	--	0.734	--
199	pentadecanal	1711	B	--	--	0.012
200	(z,z)-farnesol*	1713	B	--	0.076	0.092
201	methyl tetradecanoate	1726	A	t	0.033	--
202	m-tetrabutyl phenol	1727	B	t	--	0.374
203	(E,Z)-farnesol*	1742	B	--	0.234	--
204	isobutyl dodecanoate	1753	B	t	--	0.043
205	tetradecanoic acid	1780	A	1.168	1.384	t
206	(Z)-9-tetradecenoic acid*	1783	A	--	0.134	--
207	butyl dodecanoate*	1786	B	--	--	0.043
208	ethyl tetradecanoate	1793	A	0.692	1.260	1.232
209	hexadecanal*	1811	C	t	0.234	--
210	benzyl salicylate	1863	A	--	t	0.012
211	pentadecanoic acid*	1878	A	--	t	0.021
212	1-hexadecanol	1879	A	4.040	10.025	t
213	methyl linolenate	1893	A	t	--	0.098
214	propyl tetradecanoate*	1896	A	0.211	t	--
215	ethyl pentadecanoate*	1897	A	t	0.030	--
216	2-heptadecanone	1900	B	2.213	5.144	0.859
217	2-Nonadecanone*	1902	B	--	0.437	--
218	methyl hexdecanoate*	1926	A	0.145	--	--
219	phytol*	1942	B	t	0.316	--
220	(Z)-9.-hexadecenoic acid*	1953	B	--	0.904	--
221	butyl tetradecanoate*	1986	B	0.030	--	--
222	hexadecanoic acid	1991	A	1.877	4.612	1.215
223	ethyl hexadecanoate	1993	A	1.865	4.029	0.543
224	isopropyl hexadecanoate	1999	B	t	--	0.032
225	propyl hexadecanoate*	2091	B	0.065	t	--
226	methyl linoleate	2093	A	t	0.090	--
227	methyl octadecanoate	2128	A	--	--	0.012
228	isobutyl hexadecanoate	2135	B	t	t	--
229	oleic acid	2141	A	--	0.043	--
230	ethyl linoleate*	2159	A	t	--	0.042
231	ethyl linolenate	2169	A	0.021	t	--
232	octadecanoic acid	2172	A	t	0.290	--
233	ethyl oleate	2179	A	t	--	0.036
234	butyl hexadecanoate*	2188	B	0.012	--	0.023
235	ethyl octadecanoate	2193	A	--	t	0.740
236	isopentyl hexadecanoate	2260	B	0.013	t	--
237	propyl octadecanoate*	2296	B	0.021	--	t
238	butyl octadecanoate	2388	B	t	--	--
239	pentacosane*	2500	B	t	1.492	t
240	nonacosane*	2900	B	1.074	t	t
Total identified compounds (%)				**97.739**	**95.232**	**98.209**

M.Z.: *Mangifera indica* cultivar zebdeya; **M.H.**: *Mangifera indica* cultivar hindi; **M.C.**: *Mangifera indica* cultivar cobaneya. The reliability of the identification proposal is indicated by the following: **A**, mass spectrum and Kovat's index agreed with literature data (Adams; 2009); **B**, mass spectrum agreed with mass spectral NIST database, Mass Finder database (G1036A, and Wiley7 Mass Finder. *: Reported for the first lime in mango, t: < 0.01%, --: not detected.

(0.220%), γ-eudesmol (0.212%), α-muurolol (0.548%), α-cadinol (0.434%), cadalene (0.290%), ethyl tridecanoate(0.230%), 2-pentadecanone (0.734%), (E,Z)-farnesol (0.234%), (Z)-9-tetradecenoic acid (0.134%), hexadecanal (0.234%), 2-Nonadecanone (0.437%), phytol (0.316%), (Z)-9.-hexadecenoic acid (0.904%), octadecanoic acid (0.290%) and pentacosane (1.492%) in *M. indica* cultivar hindi while, 2,3-pentanedione (0.174%), 2-heptanone (1.062%), α-pinene (5.579%), 2-carene (0.121%), α-terpinene (0.216%), limonene (5.575%), acetophenone (0.121%), p-tolualdehyde (0.211%), p-cymenene (0.151%), ethyl heptanoate (0.133%), isophorone (0.234%), neo-allo-ocimene (2.267%), borneol (0.114%), α-terpineol (0.134%), cuminaldehyde (1.032%), p-anisaldehyde (0.174%), bornyl acetate (1.032%), undecanal (0.134%), γ-nonalactone (0.210%), germacrene D (0.798%), viridlflorene (1.052%), β-sesquiphellandrene (0.170%), methyl dodecanoate (0.121%), δ- cadinene (0.497%), germacrene B (0.798%), ethyl dodecanoate (2.374%), humulene epoxide II (5.579%), ar-turmerone (5.579%), m-tetrabutyl phenol (0.374%), ethyl hexadecanoate (0.543%) and ethyl octadecanoate (0.740%) %) in *M. indica* cultivar cobaneya. (Table 1, Figure 1)

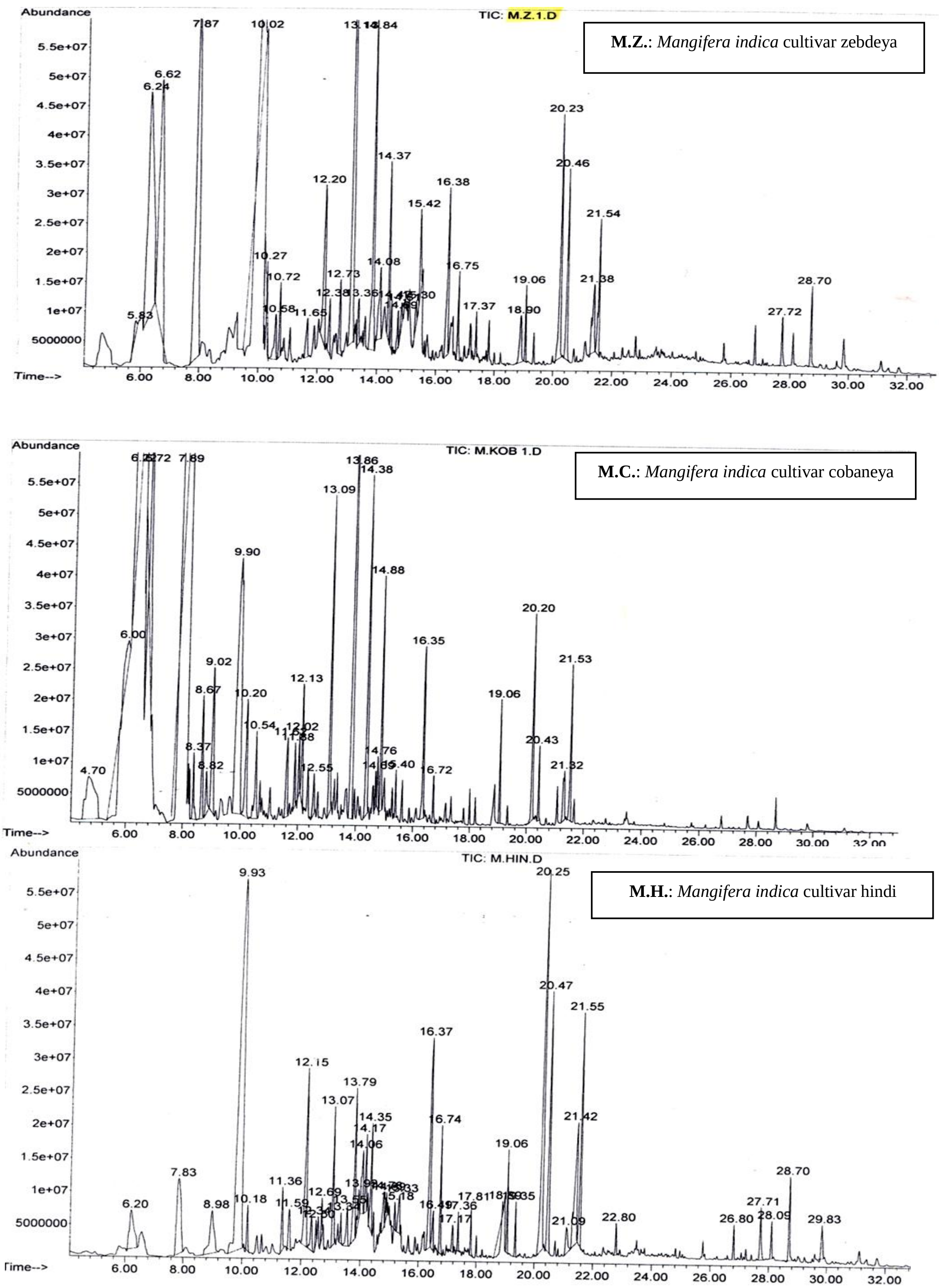


Figure 1. GC chromatograms of *Mangifera indica* cultivars **(M.Z.**: *Mangifera indica* cultivar zebdeya; **M.H.**: *Mangifera indica* cultivar hindi; **M.C.**: *Mangifera indica* cultivar cobaneya).

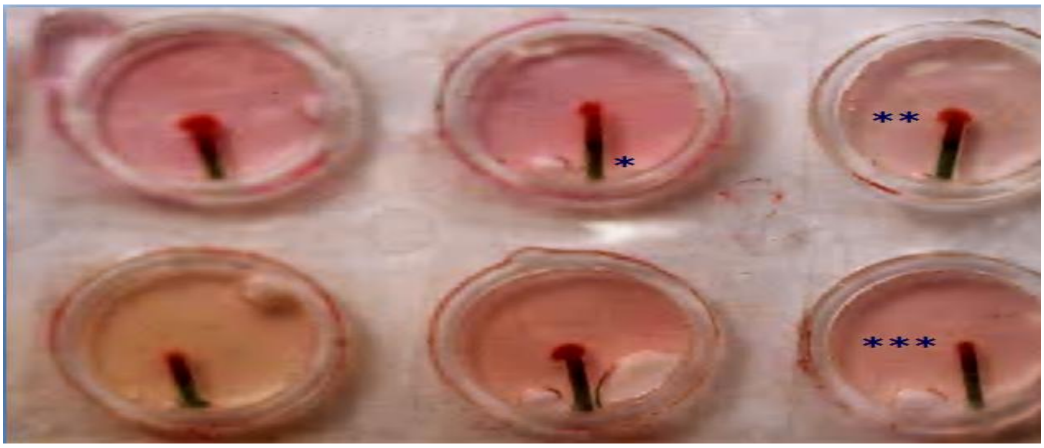
4.2. Immunostimulant activity:

Despite many published reports dealing with bioactivity of compounds isolated from *Mangifera* species little was known about its immunostimulant activity prior to our investigation.

Non-specific immune response using "Macrophage migration index" showed the lowest macrophage migration index with *Echinacea purpurea* followed by *M. indica* cultivar zebdeya in all concentrations tested, *M. indica* cultivar cobaneya at concentrations 500-2000 µl/ml and *M. indica* cultivar hindi at concentrations 2000 µl/ml only. So, peel essential oils of *M. indica* cultivar zebdeya and *M. indica* cultivar cobaneya showed appreciable non specific immunostimulant activity (low Macrophage migration index) comparable to the results displayed by *Echinacea purpurea*. (Table 2)

Table 2. Macrophage Migration Inhibition Index of essential oils of *Mangifera indica* cultivars

Conc. (µl/ml)	*M. indica* cultivar zebdeya	*M. indica* cultivar hindi	*M. indica* cultivar cobaneya	*Echinacea purpurea*
100	*0.079±0.012	0.131±0.063	0.152±0.098	*0.026±0.007
500	*0.072±0.016	0.187±0.034	*0.042±0.011	*0.025±0.011
1000	*0.070±0.043	0.128±0.033	*0.042±0.008	*0.019±0.012
1500	*0.061±0.011	0.106±0.043	*0.035±0.010	*0.018±0.013
2000	*0.049±0.013	*0.067±0.009	*0.053±0.012	*0.015±0.023



The results were presented as mean ± S.E. Student T test was used for the statistical analysis of data.
*: Results with $p < 0.01$ were considered as statistically significant

Figure 2: Migration inhibition of albino mice spleen cells from capillaries in a multi-chamber disposable plate (Sterillin). Each well contains one replicate of each test which is randomly distributed. The inhibited migration is clearly shown.
*: Capillary tube containing macrophages. **: Migration of macrophages from the Capillary tube to the surrounding culture media. ***: Complete inhibition of migration of macrophages from the capillary tube to the surrounding culture media.

Regarding the immune response using "phagocytic index", it was apparent that *M. indica* cultivar zebdeya and *M. indica* cultivar cobaneya had higher phagocytic indices up to 1.47and 1.06 respectively compared to *E. purpurea* that had a phagocytic index of 1.38 at concentration 2000 µg/ml. (Table 3)

Table 3. Phagocytic Index of essential oils of *Mangifera indica* cultivars

Conc. (µl/ml)	*M. indica* cultivar zebdya	*M. indica* cultivar hindi	*M. indica* cultivar cobanya	*Echinacea purpurea*
100	0.59±0.09	0.22±0.05	0.62±0.05	0.46±0.05
500	*0.96±0.05	0.28±0.03	0.30±0.01	0.36±0.09
1000	*1.35±0.08	0.37±0.07	0.46±0.02	0.56±0.04
1500	*1.47±0.23	0.37±0.11	0.47±0.05	*0.92±0.08
2000	*1.38±0.15	*0.77±0.08	*1.06±0.07	*1.38±0.23

The results were presented as mean ± S.E. Student T test was used for the statistical analysis of data.
*: Results with $p < 0.01$ were considered as statistically significant **.**

4.3. Anticancer activity:

The cytotoxicity of the essential oils were studied *invitro* against MCF-7, HCT-116 and Hep-G2 cancer cell lines at different concentration (0.5, 5.0, 10.0, 20 µl/ml). The results obtained from MTT assay after 48 hrs of incubation showed significant effect on MCF-7, HCT-116 and Hep-G2 with IC_{50} = 1.62-1.77, 2.95-5.56 and 2.76-3.14 µl/ml for respectively. Interestingly, *M. indica* cultivars zebdeya, cobaneya and hindi expressed a valuable cytotoxic effect on MCF-7 breast carcinoma cell line as compared to standard antitumor drug doxorubicin as it led to inhibition in cell growth as concluded from IC_{50} values 1.62-1.77µl /ml as shown in (Table 4).

Table 4: IC_{50} of essential oils *Mangifera indica* cultivars with different cell lines

Tumor cell line	Breast carcinoma cell line (MCF-7)	Colon carcinoma cell line (HCT-116)	Liver carcinoma cell line (HEP-G2)
Plant		**IC_{50} (µl/ml)**	
M. Z.	1.62	3.53	2.76
M.H.	1.77	2.95	3.14
M.C.	1.62	5.56	2.45

M.Z.: *Mangifera indica* cultivar zebdeya; **M.H.**: *Mangifera indica* cultivar hindi; **M.C.**: *Mangifera indica* cultivar cobaneya.

Doxorubicin [IC_{50} (0.42µg/ml for **(MCF-7)**, 1.43µg/ml for **(HCT-116)** and 1.12µg/ml for **(HEP-G2)]**

4.4. Antimicrobial activity:

In the present study, based on previous reports we have found that among the essential oils *M. indica* cultivars showed wide range of antibacterial and antifungal activities. Generally, essential oils of the *M. indica* cultivars showed a moderate to high antibacterial activity with *M. indica* cultivar hindi being the most active showing broad spectrum activity against Gram-positive (18-21 mm) and Gram-negative bacteria (16-19 mm). These results comply with those reported by Abdalla, *et al.* (2007). It also showed significant antifungal activity against *Candida albicans* (16 mm); however, data revealed that all *tested cultivars* was ineffective and have no antifungal activity against *Aspergillus flavus.*

Table 5: Antimicrobial activity of essential oils of *Mangifera indica* cultivars

Microorganism	Gram reaction	Inhibition zone diameter (mm)				
		Standard		M.Z.	M.H.	M.C.
		Gentamycin	Amphotericin B			
Bacillus subtilis **(ATCC 6051)**	G^+	32	--	15	18	15
Staphylococcus aureus **(ATCC 12600)**	G^+	31	--	14	21	15
Escherichia coli **(ATCC 11775)**	G^-	33	--	16	19	16
Pseudomonas aeruginosa **(ATCC 10145)**	G^-	30	--	13	16	15
Aspergillus flavus **Link (ATCC 204304)**	Fungus	--	16	≤6	≤6	≤6
Candida albicans **(ATCC 26555)**	Fungus	--	18	12	16	14

M.Z.: *Mangifera indica* cultivar zebdeya; **M.H.**: *Mangifera indica* cultivar hindi; **M.C.**: *Mangifera indica* cultivar cobaneya. Results are average of three replicate tests. The antimicrobial activity was done against six microorganisms by agar disc diffusion method.

Table 6: Minimal Inhibitory Concentration (MIC) of essential oils *Mangifera indica* cultivars against bacterial and fungal strains.

Tested microorganisms	M.Z.	M.H.	M.C.
		MIC (µl/ml)	
Bacillus subtilis **(ATCC 6051)**	116	85	166
Staphylococcus aureus **(ATCC 12600)**	195	74	121
Escherichia coli **(ATCC 11775)**	123	58	172
Pseudomonas aeruginosa **(ATCC 10145)**	140	89	159
Aspergillus flavus **Link (ATCC 204304)**	ND	ND	ND
Candida albicans **(ATCC 26555)**	142	78	142

M.Z.: *Mangifera indica* cultivar zebdeya; **M.H.**: *Mangifera indica* cultivar hindi; **M.C.**: *Mangifera indica* cultivar cobaneya.
ND; Not done, as essential oil (s) has no antimicrobial activity on this microorganism.

The **MIC** of the three *M. indica* cultivars essential oils (Table 6) revealed that although essential oils didn't show antimicrobial activities against *Aspergillus flavus* the peel essential oils demonstrated a variability in the inhibitory concentrations of each essential oil for the given microorganisms. The essential oils of peels showed activities in the range (concentrations) from 116 to 142 µl/ml for *M. indica* cultivar zebdeya, 58 to 89 µl/ml for *M. indica* cultivar hindi and from 121 to 172 µl/ml for *M. indica* cultivar cobaneya.

The lowest variation (58 µl/ml) was observed for essential oil of *M. indica* cultivar hindi on *Escherichia coli* that may be attributed to their higher oxygenated content of their essential oils. These results are in agreement with Toshida *et al*., (2000) who documented a MIC varying from 122 to198 µl/ml upon testing different concentrations of *M. indica* cultivars on both Gram-negative and Gram-positive bacteria.

Conclusion and future aspects

The use of plant essential oils from common waste products is an inexpensive, easily scaled up and environmentally benign. It is especially suited for making eco-friendly chemotherapeutic agents that must be free of toxic contaminants as required in therapeutic applications.

It is known that the by-products of some vegetables and fruits represent an important source of sugars, minerals, organic acid, dietary fiber and phenolics that have a wide range of action, which includes antitumor, antiviral, antibacterial, cardioprotective and antimutagenic activities. Thus new aspects concerning the use of the wastes therapeutically are very attractive. The present investigation focused on the possibility of using mango peel waste as a source of low-cost natural immunostimulant, anticancer and antimicrobial. *M. indica* peel, usually a waste product which is thrown into the environment has a very good immunostimulant, anticancer potentiality as well as the demonstration of broad spectrum of antibacterial activity by *M. indica* peels may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the treatment of various infections caused by microbes. These are novel, natural and economic sources of antimicrobics, which can be used in the prevention of diseases caused by pathogenic microbes.

Our results suggest that with the aid of plant wastes, conditional chemotherapeutic agents may have even broader range of applications in the future. Thus, a study of the exact mechanism by which essential oils inhibit signaling cascades responsible for the development and progression of the disease would be a tremendous breakthrough in the field of aromatherapy and make these agents an effective alternative in tumor and angiogenesis-related diseases.

In conclusion, *M. indica* cultivars essential oils can be used as an inexpensive source for the treatment of many infectious diseases caused by the bacteria and immune stimulation especially for cancer patient with special focus on breast cancer. Further research on the use of other botanicals can be rewarding to pursue in hunt for new herbal therapeutic agent. Therefore, this study will definitely open up as a scope for future utilization of the waste for therapeutic purpose.

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Conflict of interest

No declarations of interests.

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