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A Study on Anti-Oxidant, Anti-Bacterial, Antifungal, And Anti Platelet Aggregation Potential and Characterization of Ethanolic Extract of *Citrus limon (L) Burm. f. Rutacae* Peel from Coimbatore

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Abstract

Therapeutic medicinal plants are the significant constituents of traditional medicine preparations from antiquated occasions. A dominant part of individuals leans toward herbal-based medicines when contrasted with that of regular medication. Safe, effective and inexpensive indigenous remedies have become more popular among the people of both the urban and rural areas of India. Therefore, medicinal plants have become the essential part of the human health care system. Moreover, medicinal plants have attained more attention because of their effectiveness, increased cost of current medicines and cultural preferences. Citrus is presumably place most significant role in commercial and agricultural activities in the world. The peel of the Citrus fruit is a good source of flavanones and numerous polymethoxylated flavones, which are exceptionally uncommon in different plants. The Citrus compounds not just play a significant physiological and natural part but on the other hand, they act as a business intrigue which results in their huge number of utilization in the food and pharmaceutical ventures. Citrus limon (L) burm. f. (lemon) is to be healed of various diseases, and it has various therapeutic, well-being and dietary advantages. Numerous people thought this as a common plant and unaware of its medicinal properties and applications. By this study, we created an aware to grow country based Citrus species and their anti-platelet activity against cardiovascular disease. In this research, pharmacognostical studies in Citrus limon (L) burm. f. has been proved due to the presence of phytoconsituents of the peels.

Index Terms

Citrus Limon, Peel Extract, Biological Activity, Potential and Characterization, Nutraceuticals.

INTRODUCTION

Medicinal plants have become the most important part of human therapeutic services nowadays. Endangered medicinal plants have achieved more consideration on account of their viability, e xpanded e xpense of current medicines and social inclinations. Enormous number of plants has been accounted for to have antimicrobial and cell reinforcement potential. Large number of plants has been reported to possess antimicrobial and antioxidant potential [1]. The search for plant-based potent antimicrobials has dramatically increased because of the emergence of multiple drug resistance [2]. Identification of plant based antioxidants is another aspect which has gained immense importance to protect the cell/tissues from the damage caused by free radicals. Phenolic compounds present in plants act as powerful antioxidants which can protect the cellular machinery from free radicals by acting as hydrogen donors and radical scavenger [3]. Antioxidants act as free radical scavengers and are thus helping to mitigate the effect of oxidative stress in a variety of diseases such as cardiovascular diseases, Parkinson's disease, Alzheimer's disease, cancerogenesis, Neuro-degenerative, nephrotoxicity, diabetes and the ageing [4].Many studies have demonstrated the efficacy of plant derived products as a good source of antioxidants against various diseases induced by reactive oxygen species. Several studies have reported that phenolic compounds, such as flavonoids and phenolic acids present in plants are responsible for their antioxidant nature [5].Therefore, there is need to carry out a screening of the plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their active constituents. Lemons have antioxidant properties, so lemon juice is often added to fresh fruit that prevent oxidation and browing. It is considered to be highly antibacterial and is consumed for improving digestion. The peel is also eaten as a remedy for internal parasites and worms. It is widely used in Ayurveda. Medicinal oils of lemon may be used in aromatherapy. Lemon oil aroma does not influence the human immune system, but may enhance mood. The low pH of juice makes it antibacterial and in India, the lemon is used in Indian traditional medicines [6]. Therefore, the current study was focused to evaluate the antimicrobial, antioxidant and antiplatelet aggregation potential of fruit peel from fruits of *Citrus limon (L) burm. f* and to promote the utilization of fru it peel in therapeutics.

MATERIALS AND METHODS

Collection of Plant Material

The plant sample of *Citrus limon (L) burm.f. Rutacae* was collected from my grandmother's house in Gounder Mills, Coimbatore district, Tamil Nadu, India.

Experimental Procedure

The fresh fruits of *Citrus limon (L) burm. f.* were collected and washed in tap water. It is then again washed with distilled water. All the fruits of *Citrus Limon (L) burm. f.* were of eating quality, without blemishes or damage. The fruits were manually peeled. The tissue was removed pericarp region (peel), which includes the epicarp and mesocarp. The material was dispensed in paper bags and dried at ambient conditions in order to decrease their humidity from 85% to 10% of mass. Dried lemon peels were stored at ambient conditions before any further treatment. Afterward the peels were ground and sieved using vibrating metal sieves with mesh sizes of 2.5mm 2mm and 1mm. The *Citrus limon (L) burm. f* peel powder was showed in the figure 2.

Figure 2: *Citrus limon (L) burm. f* peel powder

Taxonomy

Kingdom: Plantae Subkingdom: Tracheobionta Class: Magnoliopsida Subclass: Rosidae.

Order: Sapindales Family: Rutaceae Genus: *Citrus*

Species: *Citrus* ×*Limon* (L.) Burm.f.

Extraction

Ethanol extraction is one of the efficient solvent e xtraction methods that are used to e xtract fragrant compounds straight from dry raw materials and impure oils. 100g of peel powder was taken in air t ight container and 200ml of ethanol was added in the method of **decoction** and this mixture was kept for 48 hours in dark and it was filtered. The sample was dark brown in color. Sample was centrifuged at 10000 rpm for 10 minutes. Before adding the samples, must weigh the empty petri dishes and dried out for two to three days. After three days, with the help of the backside of spatula it can be scraped and transferred it into petri plate. Then it can be stored under 40◦ C (not more than that) in hot air oven for 3 hours. It was again dried out for two days and kept in hot air oven for minimum 5 hours. The next day it was weighed about 1g per 10ml concentration ethanol was added and centrifuged at 10000 rpm for 15 minutes. The extracts were filtered with what Mann No.1 filter paper and stored in room temperature for further studies.

Antioxidant Acti vity

DPPH radical scavenging acti vity

DPPH radical scavenging activity was adopted from those previously described with slight modifications **[7]**. Various concentrations (100-500 µg/ mL) of samples and (3, 5-di-tert-butyl-4-hydroy-toluene) BHT (500µg/ mL) were taken in different test tubes. The volume was adjusted to 500 µl by adding methanol. 5 mL of 0.1 mM methanol solution of DPPH were added to these test tubes and vortexed. The tubes were allowed to stand at room temperature for 20 minutes. The control was prepared as above without any extract and methanol was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm. The inhibition percentage was calculated using the following formula:

% Radical scave nging acti vi ty = [(Abs orption concentration – Absorbance sample)]/ (Abs con)]×100

Where, Absorbance concentration is the absorbance of control; Absorbance sample is the absorbance of test sample e xtract/standard. (3, 5-di-tert-butyl-4-hydroy-toluene) BHT was taken as reference standard. The percentage inhibition versus concentration was plotted.

Hydroxyl radical** scavenging **assay (Deoxyribose assay)

Deo xyribose is o xid ized when e xposed to hydroxyl radicals; such degradation can be detected by heating the products in the presence of thiobarbituric acid under acidic conditions, which leads to development of a pink chromogen, Halliwell (1987), with modifications. The assay mixtures, containing the sample, used a final volume of 1 mL, (i.e.) 1 mM in deoxyribose, 24 mM in sodium phosphate (containing 15 mM Sodium chloride (Na Cl), pH 7.4), 0.1 mM in Iron (III) Chloride (Fe Cl3), 0.1 mM in Ethylenediaminetetraacetic acid (EDTA), 1 mM in Hydrogen peroxide (H2O2), and 0.1 mM in ascorbic acid. After incubation at 37 ◦C for 1 h, color development was promoted via addition of 1.5 mL of 28% (w/v) Tricarbo xy lic Acid and 1.0 mL of 1% (w/v) *thiobarbituric acid* (TBA) in 0.05M Sodium Hydro xide, followed by heating at 100 ºC for 15 min. Inhibition of deo xyribose degradation was expressed as percent decrease in absorbance, when compared to

the BHT (3, 5-d i-tert-butyl-4-hydroy-toluene) control (assay without sample).

(%) = (A control – A sample / A control) x 100.

Where, A control is the absorbance of the control and A sample is the absorbance of the test which is compared to that of the reference standard BHT (3, 5-di-tert-butyl-4-hydroy-toluene).

Anti-Bacterial & Anti-Fungal Activity

The antibacterial and antifungal activity of *Citrus limon (L) burm. f.* of crude e xtracts was determined by Agar Well Diffusion method **[8]**. Antibacterial activity was performed using selected bacterial species viz., *Escherichia* coli, *Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae,* and *Staphylococcus aureus.*

Antifungal assay was determined using various fungal species were selected viz., *Saccharomyces cerevisiae, Aspergillus species, and Candida species.*

Media and Culture** Condition **for Anti-Bacterial Acti vity

Mueller-Hinton Agar (MHA) and Nutrient Broth (NB) were used throughout the study for determining the antibacterial activity. Mueller-Hinton Agar (MHA) and Rose Bengal Agar (RBA) were used throughout the study for determining the antifungal activity. The both media was adjusted to the pH 7.0 and autoclaved at 121 °C for 15 to 30 minutes.

Preparation of the bacterial inoculums

Stock cultures were maintained at 4 °C on nutrient agar. Active cultures for e xperiments were prepared by transferring a loop of inoculums from stock cultures to conical flask of 50ml nutrient broth. Bacterial cultures were incubated with agitation for 24 hours and at 37 °C on shaking incubator. Each suspension of test organism was subsequently stroke out on nutrient agar media. Bacterial cultures then incubated at 37 °C for 24 hours. A single colony was transferred to nutrient agar medium were incubated at 37 °C for 24 hours. These stock cultures were kept at 4 °C. For use in experiments, a loop of each test organism was transferred into 50ml nutrient broth and incubated separately at 37 °C for 18-20 hours for bacterial culture.

Preparation of the** fungal **inoculums

Stock cultures were maintained at 4 °C on Rose Bengal Agar. Active cultures for e xperiments were prepared by transferring a loop of inoculums from stock cultures to petri plates of 20 ml Rose Bengal Agar fungal cultures were incubated at 27 °C for 3-5 days on shaking incubator. Each suspension of test organism was subsequently stroke out on Rose Bengal Agar media. Fungal cultures then incubated at 27 °C for 3-5 days. A single colony was t ransferred to Rose Bengal media. Agar media slants were incubated at 37 °C for 24 hours. These stock cultures were kept at 4 °C. For use in experiments, a loop of each test organism was transferred into saline water (100 ml of distilled water and 0.9 Sodium Chloride) for the further uses.

Procedure for Anti-Bacterial and Anti-Fungal Activity

MHA plates were prepared by pouring 20ml of molten media into sterile petriplates. After solidification of media, 20-25µl suspension of bacterial inoculums and fungal inoculums was swabbed uniformly in a separate petriplates. Using a sterile cork borer five wells were made in the agar medium. The e xt racts of *Citrus limon (L) burm. f.* were introduced into the wells accordingly (10µl, 25µl, 50µl, 75µl, 85µl, 1ml) and all the plates were incubated at 37◦ C for 24 hours for bacterial inoculums and 48-62 hours for fungal inoculums. Assay was carried into triplicates and control plates were also maintained. The tested cell suspension was spread on a Mueller-Hinton Agar plate. Sensitivity of the organism was determined by measuring the diameter of the zone of inhibition.

Anti-platelet aggregation activity

Anti-platelet aggregation activity was determined by the method described by **Ibrahim T *et al.* (2013**)

with some modifications **[9].** The compounds were separately solubilized in dimethyl sulfoxide (DMSO) before making up the volume with 50mMTris –HCl buffer (pH 7.4; containing 7.5 mM ethylenediaminetetra-acetic acid (EDTA) and 175mM Sodium Chloride (Na Cl) to a final 1% Dimethyl sulfo xide (DMSO) concentration. Different concentrations (20, 40, 60, 80 and 100 µg/ mL) of ethanolic e xtract of peel of *Citrus limon* were used in the assay. The platelet aggregation inhibitory activity of the ethanolic e xt ract and compound was separately evaluated on (Adenosine diphosphate) ADP (5 mM) induced aggregation. The platelets (100 µL) were pre-incubated for 5 min with different concentrations of the e xt ract and compounds before introduction of platelets agonist (20 µL) to the mixture. Aggregation was determined with the Biotek plate reader (ELx 808 UI, Biotek Instrument Supplies) using Gen5 software by following change in absorbance at 415 nm. DMSO (dimethyl sulfoxide) (1%) was used as negative control and asprin was used as positive control. The inhibitory effect of the compounds on each parameter was calculated as:

$$\textbf{Inhibition (\%) = [(A}_0 - A_1)/A_0 \textbf{ x100]}$$

Where, A_0 is the mean slope of the control and A_1 is the mean slope of the test compound.

Gas-Chromatography-Mass Spectrometry

Analytical GC-MS was carried out on a DSQ- 2000 instrument at 70 e V and 250°C. GC column DB 35 - MS Capillary Standard Non - Polar column, dimension of 30 Mts, ID: 0.25 mm, with film thickness: 0.25 µm. The in itial temperature was 70°C for 6 minutes and heated at a rate of 10°C per minute to 260°C. Carrier gas helium, flow rate 1.0 ml/ min and FID detector were used. Identification was considered tentative when it was based on mass spectral data only. The quantification of the volatile compounds was performed using percentage peak area calculations by means of a gas chromatograph, Shimadzu 2010, with a flame ionization detector (GC-FID).

Procedure

Arguslab requires a PDB format file for both ligand and receptor. The binding site was defined from the coordinates of the ligand in the original PDB file. Argusdock exhaustive search docking engine was used, with grid resolution of 0.40

Å. Docking precision was set to 'high precision' and 'flexible ligand docking' mode was employed for each docking run. Open "Arguslab Software" .Click "File" option and "Open" the Structure of the Protein we have already saved in .pdb format. Click the Structure of the protein and check whether the "Water" molecules are present or not. If "Water" is present delete all. Then click on to any "Amino Acid" in "Residue" and select it. Click " Edit" and select "Hide Unselected" and make the molecule to center of the window. Right click on to the selected Amino acid and "Make a Group from this residue". Then Make this Structure as "Binding Site". Open the drug molecule saved as .mol format using "File" option. "Make a Ligand from this Residue" by selecting it. Click "Make Surfaces" from Toolbar menu. Click "Calculation" and select "Dock a Ligand". Click "Start" and calculate the poses and energy. Save the molecule as .agl files. The receptor is treated as a rig id body and a grid potential is used to evaluate the scoring functions. This simplification allows one to perform docking more efficiently, which is especially crucial in database screening.

RESULTS

Antioxidant Acti vi ty

In the present study, antioxidant activity of the e xt ract was estimated by the following methods:

- DPPH radical scavenging activity
- H_2O_2 scavenging activity

DPPH radical scavenging acti vity

Ethanol e xtracts of the peel *Citrus limon (L) burm. f.* significant antio xidant activity by exh ibit ing DPPH free radical scavenging property. Ethanol extracts of the peel *Citrus limon (L) burm. f.* e xhib ited antioxidant activity on DPPH radicals at different concentrations, using BHT as standard antioxidant. The DPPH radical scavenging activity was showed in the figure 3.

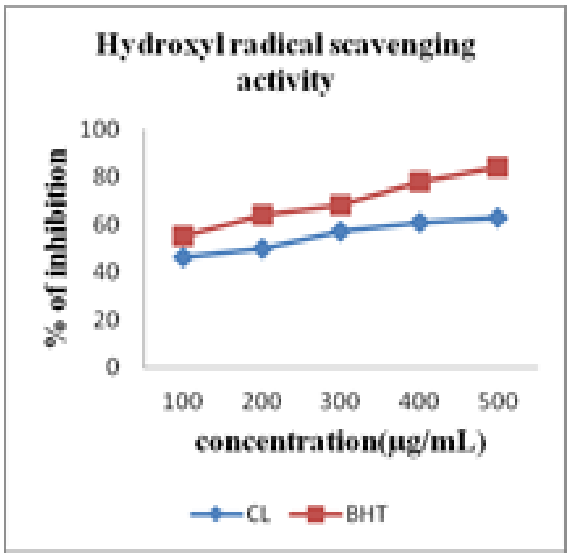

Figure 3: The radical scavenging activity of *Citrus limon (L) burm. f.* by DPPH method.

Figure 3 shows the *in vitro* antioxidant potential of ethanol e xtract of *Citrus limon* at different concentrations. The results shows the maximum DPPH scavenging activity in *Citrus limon* appeared to be potent as standard BHT with inhibition of 25%, 30.89%, 46.26%, 60.91%, 71.12% of 100, 200, 300, 400 and 500µg/ml concentrations of peel extracts. These activities are lesser than BHT. Composition of essential oils and antioxidant activity of peel ethanol e xtract were analyzed in 18 local lemon cultivars. Essential oils composition was determined by GC/ FID analysis and antioxidant activity with the ABTS method. Fruit weight, polar and equatorial diameters, peel thickness, seed number, juice percentage, titratable acidity and juice pH were also determined for each cultivar. The main component in the peel essential oil was limonene, accounting for 72.5– 76.4%, followed by β-pinene (11.6– 18.7%). Several other monoterpene hydrocarbons were also identified at appreciable contents, namely terpinene (2.88–8.26%), α-pinene (1.4– 1.5%) and myrcene (0.95–1.12%). Peel ethanolic e xtracts showed a high radical scavenging power **[10].**

H2O2 scavenging activity (Deoxyribose assay)

The ethanol e xtract of *Citrus limon* by H_2O_2 scavenging activity was showed in the figure 4.

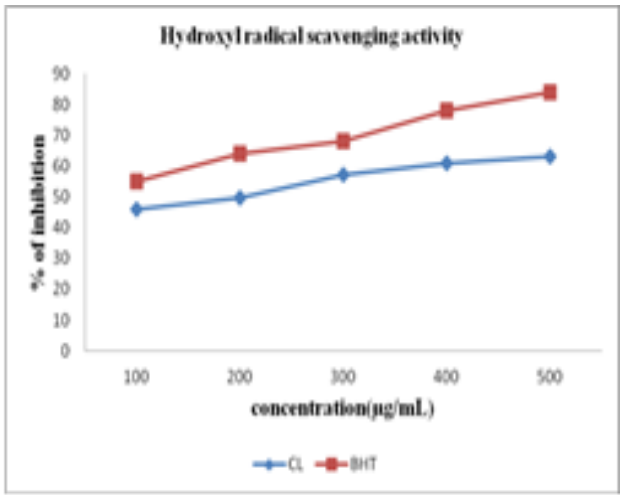

Figure 4: The scavenging activity of *Citrus limon (L)burm. f.* by H_2O_2 scavenging activity

Figure 4 shows the *in vitro* antioxidant potential of ethanol e xtract of *Citrus limon* at different concentrations. The results shows the maximum H_2O_2 scavenging activity in *Citrus limon* appeared to be potent as standard BHT with inhibition of 46.08%, 49.76%, 57.14%, 60.82%, 63.13% of 100, 200, 300, 400 and 500µg/ml concentrations of peel extracts . These activities are lesser than BHT. Antioxidant compounds present in the extracts/standard can donate electrons to H_2O_2 and converted to H_2O. Extracts of *Citrus spices* showed promising antioxidant activity for peel ethanolic to lemon. The antioxidant activity of *Citrus* fruits and their roles in the prevention and treatment of various human chronic and degenerative diseases have attracted more

and more attention. *Citrus* fruits are suggested to be a good source of dietary antioxidants **[11].** H_2O_2 scavenging activity (Hydrogen peroxide inactivates a few enzymes directly, usually by oxidation of essential thiol group (-SH). Responsible for various to xic effects can cross membranes and reacts with Fe 2+ and Cu2+ ions to form hydro xy radical. Antioxidant compounds react with H_2O_2 and converted into H_2O. Quantitative phytochemical indicated that the plant contains significant amounts of phenolic compounds were res possible for antioxidant and free radical scavenging effect of plant material **[12].**

Antibacterial and Anti-Fungal Acti vity

The peels of *Citrus limon* was evaluated for their antibacterial potential against the five bacteria namely ***Klebsiella pneumonia, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli*** by agar well diffusion method. Different solvent extracts showed antibacterial activity against these selected bacteria. The ethanolic e xtract of the *Citrus limon* showed a maximum antibacterial activity against the tested pathogens. But comparatively .the ethanolic e xt ract of the *Citrus limon* showed a minimum antibacterial activity against Escherichia coli.

Antifungal Acti vity

The peel e xtract of *Citrus limon* was evaluated for their antifungal potential against the three fungal organisms namely *Saccharomyces cerevisiae*, *Candida species*, *Aspergillus species* by agar well diffusion method. Different solvent extracts showed antifungal activity against these selected fungus. The ethanolic e xtract of the *Citrus limon* showed a maximum antifungal activity against the tested *Saccharomyces cerevisiae*, *Aspergillus species*. The ethanolic e xtract of the *Citrus limon* showed a minimum antifungal activity against *Candida species*.Fungal infections of the skin, also known as 'mycoses' are generally mild. Superficial fungal infections affect outer layers of the skin, nails, and hair, and the main group that causes superficial fungal infections are dermatophytes (tinea), Candida, and moulds. The high sensitivity exhib ited by *C. glabrata* towards both fractions of *C. limon* partially confirms the use of *C. limon* in the management of skin diseases in traditional medicine. Numerous studies have described the inhibitory activities of *Citrus* against human pathogens, fungi, yeasts, and food pathogens **[13]**. The most important active ingredients of lemon are monoterpenes such as limonene, γ-terpinene, β-pinene, and the aldehydes geranial and neral**[14]**.

Anti-Platelet Aggregation Acti vity

There was significant inhibition in platelet aggregation at moderate dose induced by adenosine diphosphate (ADP). The percentage inhibition of platelet aggregation was found to be 6.18%, 23.91%, 47.48%, 50.67%, 75.78% for *Citrus limon* peel e xtracts. The IC value for the percentage inhibition of platelet aggregation were determined for a concentration range 20, 40, 60, 80, 100 µg/ ml. *Citrus limon* indicated the higher yield of the platelet aggregation. The antiplatelet aggregation activity was showed in the figure 5.

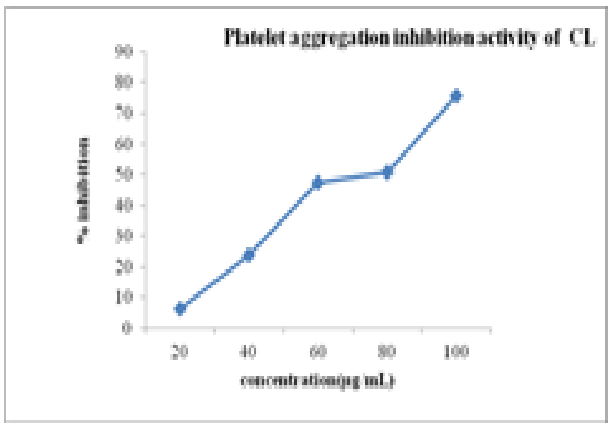

Figure 5: Antiplatelet aggregation activity of *Citrus limon*

P2Y$_{12}$ displays an elevated basal activity in vitro, and as such, inverse agonists may be therapeutically beneficial compared with antagonists**[15].**

Gas-Chromatography – Mass Spectrometry

The ethanolic peel e xt ract of *Citrus limon (L) burm. f.* which has high *in vitro* activity were subjected to GC-MS In present investigation total **three hundred** bioactive chemical constituents are identified in ethanolic e xtract of *Citrus limon (L) burm. f.* with important properties. The GC-MS chromatogram of ethanolic e xtract of *Citrus limon (L) burm. f.* is shown in the figure 6.

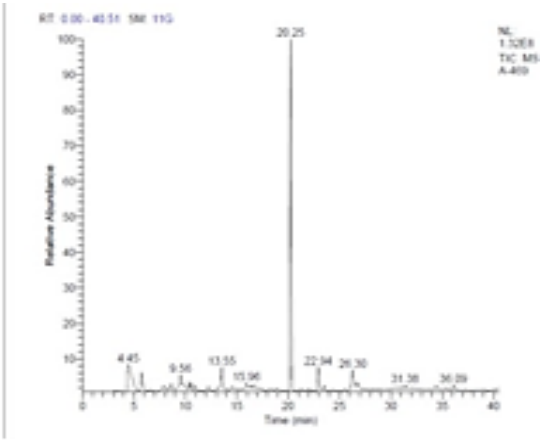

Figure 6: GC-M S chromatogram of ethanolic e xtract of Citrus *limon (L) burm. f.*

Library Search Compounds Results

The GC-MS chromatogram library search compounds results of ethanolic e xtract of *Citrus limon (L) burm. f.* is shown in table 1.

Based on our findings, the main constituents of the lemon peel were Citric acid (7.28%), ally lo xy methylnitrile (2.80%), Dodecanoic acid (CAS) (2.57%), Glycyl-D-asparagine (1.47%), 2-Furancarbo xaldehyde, 5-(hydro xy methyl)- (CAS) (1.21%) to be found. The peel and leaf oils of *Citrus limon L.* fro m Benin were analyzed by

capillary GC on two columns of different polarity, and by GC/MS. In these oils 42 and 27 components were identified, representing over 99.7% of the oils. The main constituents of the lemon peel oil were limonene (70.4%), γ-terpinene (11.8%) and β-pinene (4.2%). The leaf oil consisted mainly of limonene (40.8%), β-pinene (18.5%) and citronellal (16.5%) **[16].**

Molecular Docking

According to the GC- MS results the retrieved compounds were identified by molecular docking. There are about **300** compounds. From this, the high probability compounds were docked.Docking allows virtually screening of compounds and predicts the strongest binders based on various scoring functions. It e xplo res ways in which two molecules such as drug and a protein receptor $P2Y_{12}$ and Limonene fit together and dock each other well. The compounds binding to a receptor inhibit its function and thus act as drug. P2Y12 receptor antagonists have emerged as the current mode of treatment in conjunction with aspirin for acute coronary syndromes (ACS) and for patients undergoing percutaneous coronary intervention (PCI) with stenting through their ability to inhibit platelet aggregation. Successful platelet aggregation requires activation of both P2Y1 and P2Y12 receptors P2Y12 deficiency. Congenital P2Y12 deficiency is an autosomal recessive disorder.Clinically important thresholds to Verify Now P2Y12 test:

- High risk of bleeding with surgery: <208 Plavix reactive units (PRU) [9]
- Acceptable risk of bleeding with surgery: >208 PRU [9]
- High risk of thrombosis after stent placement: >230 PRU (resistance to P2Y12 inhibitors)
- Low risk of thrombosis after stent placement: <208 PRU (therapeutic range)

Thus the properties of the antiplatelet and antioxidant activity which is present in the p2y12, limonene was docked and treated by drugs for antiplatelet aggregation and antioxidant disorders. Limonene showed antioxidant and radical scavenging activities in several model systems and cytotoxic ity against MCF-7, K562, PC 12 [106], A-549, HT-29 cell lines **[17],** and Hep G2 hepatocarcinoma cell lines **[18].**

Table 1: Library Search Compounds Results

S.No	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
1	Citric acid	36.81	C6H8O7	192	7.28
2	ally lo xy methylnitrile	17.57	C5H7NO	97	2.80
3	Glycyl-D-asparagine	13.42	C6H11N3O4	189	1.47
4	2-Naphthol, 1-(p-chlorophenylazo)-	13.55	C10H14D4O2	170	0.29
5	2-Furancarbo xaldehyde,5-(hydroxy methyl)-(CAS)	63.69	C6H6O3	126	1.21
6	Dodecanoic acid(CAS)	20.36	C12H24O2	200	2.57
7	8-hydro xyneomenthol	13.26	C10H20O2	172	0.45
8	1,2-Cyclohe xanedio l,1-methyl-4-(1-methyl ethenyl)-	58.57	C10H18O2	170	1.05
9	1-Propene-1,2,3-tricar boxylic acid	32.83	C6H6O6	174	1.00
10	2-Isopropylidene-5-methylhex-4-ena l	29.81	C10H16O	152	0.86

Table 2: The bioactive compounds from the ethanol e xtract of Citrus limon (L) burm. f. with receptor protein P2Y12 and Limonene

S.No	Compound Name	E.VALUE (Kcal/ mol)	POS E 1	Figure Number
1	Citric acid	-5.74675 kcal/ mol	-5.45 kcal/ mol	21
2	5,Hydro xy methylfurfural	-5.69636kcal/ mol	-5.70 kcal/ mol	22
3	Glycyl-D-asparagine	-5.96263 kcal/ mol	-5.96 kcal/ mol	23
4	1,2-Cyclohe xanedio l	Atomic number 15 has no shells	-6.88 kcal/ mol	24
5	Aconitic acid	-5.86701kcal/ mol	-5.87 kcal/ mol	25
6	Isopropyl myristate	-6.76152 kcal/ mol	6.75 kcal/ mol	26

DISCUSSION

In the recent past the medicine worlds have changed their entire focus on the traditional medicine and natural cure since the side effect and toxicity of the synthetic counterpart is increasingly more. Hence the present work was undertake out mainly focused on identifying phytochemical screening, antioxidant activity, antimicrobial activity, antifungal activity, antiplatelet aggregation activity, GC-MS, molecular docking from the traditionally used well known medicinal plants. *Citrus limon (L) burm. f.* were selected for the present investigation.

Antioxidant Activity

Antioxidant activity of ethanol extract of *Citrus limon (L) burm.f.* was studied using DPPH and hydrogen peroxide

method. The e xtract showed the antioxidant activity was compared with standard BHT for free radical scavenging activity and hydrogen peroxide. The calculation made in terms of percentage inhibition and IC50 value. *In vitro* scavenging effect of both DPPH method and H_2O_2 method is observed good in the Ethanol e xt racts of *Citrus limon*. This implies that, it was a highly active in scavenging the free radicals by annealing protons thereby reducing the oxidative stress and diseases and disorders related to it.

Antibacterial & Antifungal Acti vi ty

The *in vitro* antibacterial activity was undertaken out for the agar well diffusion method using gram positive and gram negative bacteria. Ethanol e xt ract of *Citrus limon (L) burm.f.* peel showed a potent activity. This shows the extract has high *in vitro* antibacterial property. This property is beneficial in discovery of novel phytomedicine and treatment diseases. The *in vitro* antifungal activity was undertaken out for the agar well diffusion method using different fungal species. This shows that the slight change in *saccharomyces cerevisiae* and *aspergillus species* and high *in vitro* antifungal property of *candida species*. This property is beneficial in discovery and treatment diseases.

Antiplatelet Acti vity

Ethanol extracts of *citrus limon* possess good in *in vitro* antiplatelet aggregation activity. It mainly activates the $P2Y_{12}$ and Limonene receptors to be active and disease free. In silico docking approach was also applied to screen these compounds for their efficacy against selected drug targets of platelet aggregation and blood coagulation

Gas Chromatography – Mass Spectrometry

Ethanol e xtracts of *citrus limon (L) burm. f.* peel identified **three hundred** bioactive compounds. The major analysis to found the ligands in the given sample of *Citrus limon*.

Molecular Docking

In silico analysis is also shows that a compound "**Isopropyl myristate**" present in the *citrus limon* has a good docking activity with the least E value (- 6.88 kcal/ mol) when molecularly docked with human antiplatelet and also coagulative receptors of limonene and $P2Y_{12}$ using Arguslab. Top two potent compounds ($P2Y_{12}$ and Limonene) and their source herbs could be promising drugs for antiplatelet aggregation.

CONCLUSION

From the above findings it can be concluded that antioxidant, in vitro antibacterial and antifungal and antiplatelet activities may be due to the presence of phytoconsituents of the peels. Thus the fruit peel of *Citrus limon* has got a broad spectrum of antimicrobial and antioxidant activity and has a promising potential for treating several diseases.

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