

A review on Pharmacological actions of Citrus medica, Citrus aurantium and Citrus maxima

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ABSTRACT

Citrus species are one of the major cultivated crops throughout the globe and have broad economic and pharmaceutical importance. Citrus-based products are used in various food and medicinal industries due to their strong fragrance and therapeutic properties. They are rich in micro as well as macro nutrients. Analysis of their compounds has led to the identification of biologically active components which include flavonoids and alkaloids that contribute to pharmaceutical prominence. The Citrus species from family Rutaceae has worldwide applications such as cardiovascular and gastrointestinal problems. There are many reports on a wide range of activities such as anti-inflammatory, anti-oxidant, immunomodulatory, metabolic, cardiovascular and neuroprotective effects. Citrus occupies a place of considerable importance in the fruit economy of the country.

Keywords : Citrus medica, Citrus aurantium, Citrus maxima, Phytochemical properties, Pharmacological properties.

I. INTRODUCTION

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicines since prehistoric times. Plants synthesize hundreds of chemical compounds for various functions. Citrus is the most important genus in the Rutaceae family. Tanaka, a Japanese Citrus taxonomist deduced that northeastern India, northern Burma, Yunnan and the surrounding areas of China are the origin of Citrus species. Different plants of these species are used in various industries such as the food and cosmetic industry. Several parts of Citrus species are natural sources of carbohydrates, dietary fibres, water-soluble vitamins and phytochemicals such as flavonoids, limonoids and carotenoids. Traditionally, citrus

have been used to manage constipation, cramps, colic pains, diarrhoea, bronchitis, tuberculosis, cough, cold, obesity, menstrual disorder, angina, hypertension, anxiety, and depression and stress⁽¹⁾.

Rutaceae, the family of flowering plants composed of 160 genera and about 2,070 species. Rutaceae includes woody shrubs and trees (and a few herbaceous perennials) and is distributed throughout the world, especially in warm temperate and tropical regions. The family contains a number of economically important fruit trees as well as several ornamental species. Citrus fruits, including oranges, lemons, limes and grapefruits, are a principal source of important nutrients, which are suggested to be responsible for the prevention of degenerative disease. The species cultivated in India includes: Citrus aurantifolia, C. aurantium, C. deliciosa, C. grandis, C. jambhiri, C. karna, C. latifolia, C. limetta, C. limettioides, C. limon, C. limonia, C. lycopersicaeformis, C. macroptera, C. maderaspatana, C. madurensis, C. medica, C. megaloxycarpa, C. nobilis, C. paradisi, C. paratangerina, C. pennivesiculata, C. pseudolimon, C. reshni, C. reticulata, C. rugulosa, C. sinensis and C. unshiu.

The bio-activities of Citrus are due to the presence of bioactive compound such as phenolics, flavonoids, essential oil and vitamins. The composition of the fruits is affected by climate, growing conditions, various treatments, maturity, root-stock and variety⁽²⁾.

II. MATERIALS AND METHODS

The study was conducted by gathering and carefully examining a variety of research articles, novels, and other literary works, as well as taking notes in these publications.

Scientific classification

	Citrus medica	Citrus aurantium	Citrus maxima
Kingdom	Plantae	Plantae	Plantae
Phylum	Tracheophyta	Tracheophyta	Tracheophyta
class	Magnoliopsida	Magnoliopsida	Magnoliopsida
Order	Sapindales	Sapindales	Sapindales
Family	Rutaceae	Rutaceae	Rutaceae
Genus	Citrus	Citrus	Citrus
Species	Citrus medica	Citrus aurantium	Citrus maxima

Traditional uses

Plant species	Uses
Citrus medica	Sedative, antiemetic, insect repellent, diuretic and antitoxic
Citrus aurantium	Treatment of nausea, indigestion and constipation, cancer, cardio-vascular effect, sedative.
Citrus maxima	Ulcer, febrifuge, dyspepsia, lumbago, fever, cardiogenic, gastrointestinal disorders, diabetes and cardiovascular disease

Phytochemical constituents

Plant species	Chemical constituents
Citrus medica	Iso-limonene, citral, limonene, phenolics, flavonones, vitamin C, pectin, linalool
Citrus aurantium	Flavanoids, phytosterols, carbohydrates, saponins, volatile oil, tannins, terpenoids, proteins, limonoids, carotenoids, phenolic compounds, hesperidin, neohesperidin, naringin,
Citrus maxima	Alkaloids, amino acid, carbohydrate, carotenoid, coumarins, flavanoids, monoterpenes, sesquiterpenes, steroids

PHARMACOLOGICAL PROPERTIES

Citrus medica

Common name : Citron, Bijoru, Wild Lemon, Bijapura, Limbu, Nimbu

● **Analgesic Action**

In this study hot plate technique and tail immersion methods were used for the evaluation of analgesic activity of C.medica fruit decoction. The dosage used were 1, 2 and 4ml/kg. In hot plate method the three dosages showed effectiveness. However, in the tail immersion method, only the doses of 2 and 4 ml/kg were found to be effective, while the 1 ml/kg dose did not show efficacy in evaluating centrally acting drug's analgesic effects. The 4 ml/kg dose of C.medica decoction exhibited pain inhibition comparable to diclofenac sodium injection in both hot plate and tail immersion methods. This study confirms the conventional usage of C.medica decoction as an analgesic. The observed pain-relieving action of C.medica is likely attributed to its flavonoids and phenolic composites, which are known to possess analgesic properties⁽³⁾

● **Anticancer and antimutagenesis property**

This study has been tried to consider anticancer effects of half-ripe and ripe Citrus medica fruit juice on cancerous cells. The method of vital capacity test (MTT) has been used in order to consider cytotoxicity of Citrusmedica fruit juice on cancerous cell lines (in vitro) and results have been calculated in terms of stimulation index and assessed by t-test. Salmonella typhimurium TA100 used for Ames test. In this test fruit juice was added to test tube containing 0.5 ml of the overnight fresh bacterial culture, 0.5ml of histidine and biotin solution (0.5 mM histidin/0.5N biotin), 10 ml top agar (50 gr/lit Agar + 50 gr/lit NaCl), sodium azide as acarcinogen (1.5 µgr/ml Sadium azide) and then content of this tube distributed on the surface of minimum medium of glucose agar (%40 glucose), after 3 seconds of shaking incubation was performed at 37⁰C for 48 hours. Each treatment was repeated 3 times. In the test after 48 hrs incubation at 37⁰C, reversed colonies were counted in control and test plates and after angular conversion, results were compared by analysis

variance. Most materials in their original form are inactive in terms of carcinogenic effects and most materials to become metabolically active to display mutagenesis properties. The results of colony counting in Ames test under 25 µl/ml of the fruit juice (with regard to the results of vital capacity test) showed that there was a significant difference between half-ripe and ripe fruit juice antimutagenesis effect on colony growth with controls (distilled water and sodium azide)⁽⁴⁾

- **Anti-diabetic, hypocholesterolemic and hypolipidemic activity**

In this study anti-diabetic and hypolipidemic activity of petroleum ether extract of *Citrus medica* seeds in streptozotocin (STZ) induced diabetic rats were studied. The study was carried out using albino rats of either sex weighing 150–200 gm. One group was selected as control group (buffer alone) and four groups of STZ induced diabetic rats (5 in each group) were administered vehicle (1% tween 80), seed extract (200 and 400 mg/kg, p.o.) of *C. medica*. and standard drug glibenclamide (5 mg/kg) for 15 days after 10 days of single dose of STZ (60 mg/kg) intraperitoneal administration. Blood samples were collected by retro-orbital puncture and were analyzed for blood glucose, serum cholesterol, triglycerides, HDL, LDL and VLDL on days 0, 3, 10 and 25 by using diagnostic kit. The petroleum ether extract of *C. medica* seeds (200 and 400 mg/kg, p.o.) induced significant reduction ($p < 0.05$) of fasting blood glucose, serum cholesterol, serum triglycerides, LDL and VLDL in dose dependent manner after 15 days of drug administration. Though 200 mg/kg/day seed extract for 15 days was not showing any change in HDL level, while 400 mg/kg/day dose significantly increased HDL level in diabetic rats. So it is concluded that *C. medica* seeds have significant antidiabetic, hypocholesterolemic and hypolipidemic activity⁽⁵⁾.

- **Anti-ulcer activity**

In this study the anti-ulcer activity of aqueous extract of *Citrus medica* fruits were determined. It was demonstrated a significant reduction in ulcer scores, percentage of ulcers, and ulcer index in rats with ethanol-induced ulcers. The anti-ulcer outcome of *C. medica* is attributed to the existence of flavonoids among its constituents, as polyphenolic compounds are known for their antioxidant properties and gastro protective effects. These findings were further supported by histopathological observations, which revealed

reduced mucosal ulceration, reduced inflammatory infiltration in the mucosa, and less edema in the submucosa in the groups pretreated with the extract as contrasted with the untreated group. In conclusion, the fruit extract of *Citrus medica* exhibits anti-ulcer activity, thus validating its traditional use as a remedy for ulcers⁽⁶⁾

- **Antimicrobial activity**

In this study antimicrobial activity of fruit juice and ethanolic extracts of root, leaf, bark, peel and pulp of *Citrus medica* was examined against seven bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris*), two fungi (*Aspergillus flavus* and *A. niger*) and a yeast *Candida albicans* of clinical origin. The level of antimicrobial effects was established using an in vitro disc diffusion method; minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined by standard agar dilution method. All extracts and fruit juice showed varied level of antibacterial activity against one or more test bacteria. Antifungal activity was shown by only root extract and fruit juice while *C. albicans* was resistant to all tested plant samples. Broad spectrum antimicrobial activity was shown by fruit juice

(MIC <1% to 3.5% and MBC 1% to 7% v/v) and fruit pulp (MIC 25 mg/ml and MBC 30 to 75 mg/ml). Root extract was found highly potent with MIC as small as 0.5 mg/ml and MBC 1 mg/ml against *S. aureus*. Among all tested plant samples leaf and peel extracts have shown less antimicrobial activity. It is concluded that fruit juice and fruit pulp extract have shown broad antimicrobial activity while root extract was very effective against some tested microorganisms⁽⁷⁾.

- **Anthelmintic activity**

In an in-vitro investigation, the petroleum ether extracts of *Citrus medica* leaves demonstrated a dose-dependent anthelmintic action, comparable to the effects of Piperazine citrate. The plant has been established to possess anthelmintic properties contrary to Indian adult earthworms (*Pheretima posthuma*). Although the precise mechanism underlying its anthelmintic effect is yet unknown, it is thought to be connected to the parasite's suppression of glucose intake and glycogen formation. Additionally, *Citrus medica* may activate nicotinic cholinergic receptors in the worms, leading to either persistent depolarization

or hyper-polarization. Finding the compound that gives Citrus medica anthelmintic properties will require more investigation. Moreover, alcoholic extracts from the rind of *C. medica* also exhibited moderate in-vitro anthelmintic activity against human *Ascaris lumbricoides*⁽⁸⁾

- **Estrogenic activity**

In this study the estrogenic activity of petroleum ether extract of *C. medica* leaves were studied. The extract when orally administered at a dose of 400mg/kg body weight, led to an increase in uterus weight and exhibited estrogen-like activity in ovariectomized rats. It demonstrated substantial estrogenic action ($P < 0.05$) at this particular dose. This suggests that the extract from *Citrus medica* leaves could serve as an innocuous and natural resource of estrogenic action, potentially beneficial for postmenopausal women. Furthermore, the estrogenic and anti-estrogenic actions of the petroleum ether extract from *C. medica* seeds were investigated in albino rats. The results indicate the extract's potent estrogenic properties, which could be utilized as an antifertility agent⁽⁹⁾
Citrus aurantium Common name : Bitter orange, Sour orange, Seville orange, Bigarade orange

- **Cytotoxic and Anticancer Effects**

In this study, Cytotoxic properties of the polysaccharides obtained from *C. aurantium* were examined. Cytotoxic activity on human breast cancer cells (MCF-7) and lung cancer cells (HCC827) and immune-enhancement effect were examined. The results indicated that *C. aurantium* polysaccharides displayed good immune-enhancement activity by stimulating the production of tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) in RAW264.7 cells and by promoting the mRNA expression levels of inducible nitric oxide synthase (iNOS), TNF- α , interleukin-1 β (IL-1 β), and IL-6. Moreover, phosphorylated extracellular signal-regulated kinase (ERK), phosphorylated c-Jun N-terminal kinase (JNK), phosphorylated p38, and phosphorylated p65 were significantly enhanced in *C. aurantium* polysaccharide-treated RAW264.7 cells. Isolimonic acid and ichanexic acid from ethyl acetate extract of *C. aurantium* were isolated by using chromatographic methods. The compounds for their inhibitory action on human colon cancer cells (HT-29) proliferation, apoptosis, and on noncancerous (COS-1 fibroblast) cells were investigated. The compounds displayed an increment in the cell counts in G2/M stage,

demonstrating a potential effect in the cell-cycle arrest⁽¹⁰⁾

- **Anxiolytic and Sedative effects**

In this study, the anxiolytic and sedative effects of *C. aurantium* were examined by in vivo light-dark box and the marble-burying assays. *C. aurantium* essential oil enhanced the period the mice spent in the light chamber, as well as the number of transitions between the two compartments in the light-dark box test. Moreover, single and repeated treatments with the essential oil were reported to suppress marble-burying behavior. The anxiolytic activity of the blossom of *C. aurantium* was also evaluated clinically on 60 patients undergoing minor surgical operation with no organic pathology. Two hours before the anesthesia induction, two groups consisting of 30 patients were administered at 1 ml/kg of either *C. aurantium* distillate or saline solution. Anxiety was assessed by using the Spielberger state-trait anxiety inventory, the Amsterdam preoperative anxiety, and information scale. The outcome of this study revealed that *C. aurantium* could be active by reducing preoperative anxiety before minor operation⁽¹¹⁾

- **Antidiabetic Effects**

An in vivo study was performed to evaluate the antidiabetic effect and to reveal the toxicity profile of the aqueous extract of the fruits of *C. aurantium*. In this study, NMRI lean mice (6-week-old) and C57BL/6J lean mice (6- or 11-week-old) were used. A single dose, which corresponds to seventy-fold of a human daily dose, was detected to be nontoxic to the animals. A significant weight loss was determined when the dosage, which corresponds to tenfold of a human daily dose, was administered to C57BL genetic diabetic mice for 6 weeks. These mice were maintained on the carbohydrate-deficient diet during the treatment period. The food intake was not significantly different from the control group animal however, the serum triglyceride levels of the treated animals were significantly higher suggesting the lipid mobilization from internal stores. The fatty acid levels of the eyes of the treated mice remarkably reduced along with stearoyl-CoA desaturase activity. A possible effect of the extract obtained from *C. aurantium* on liver metabolism was evaluated. In order to measure catabolic and anabolic pathways, an isolated perfused rat liver was used. The extracts were found to enhance glycolysis, glycogenolysis, oxygen uptake, and

perfusion pressure. *C. aurantium* extract enhanced gluconeogenesis at low concentrations, however, inhibited at high concentrations. The effects of *C. aurantium* extract on liver metabolism was found to be similar to those of adrenergic agents⁽¹²⁾

- **Antiobesity Effect**

In this study, the effect of *C. aurantium* on obesity were examined. *C. aurantium* extract has been commonly utilized for the weight loss and as sports performance enhancer, in dietary supplements. Therefore, the use of *C. aurantium* extract and its constituent p-synephrine, for the treatment of obesity in 360 subjects, was reviewed. More than 50% of the subjects involved in these clinical studies were overweight, and approximately two-thirds of them consumed caffeine (132–528 mg/day) and p-synephrine (10–53 mg/day). Approximately 44% of the subjects used a *C. aurantium*/p-synephrine product, while the remaining consumed a combination product containing multiple ingredients with p-synephrine. The results showed that *C. aurantium* extract alone or in combination with other ingredients did not cause significant adverse effects including an increase in heart rate or blood pressure or change in electrocardiographic data, serum chemistry, blood cell counts, or urinalysis. p-Synephrine, alone or in combination products, was demonstrated to enhance metabolic rate and energy expenditure and to promote weight loss when given for 6 to 12 weeks⁽¹³⁾

- **Antimicrobial activity**

In this study the antimicrobial activity of *C. aurantium* juice against *Salmonella enterica*, *Typhimurium* and *Listeria monocytogenes*. For this purpose, both neutralized and un-neutralized juice samples with various concentrations were tested for the inoculation of the microorganisms at 4°C and 37°C temperatures, during a period of seven days. The results showed that *Salmonella enterica*, *Typhimurium* and *Listeria monocytogenes* not only survived but also grown for two days in neutralized juice at 37°C. On the other hand, on day 7, none of them survived. The results also revealed that *L. monocytogenes* was less resistant than *S. enterica*, *Typhimurium*. The low pH of *C. aurantium* juice was suggested to be responsible for its antimicrobial potential along with the duration of the incubation period as well as the temperature⁽¹⁴⁾.

In another research, the antimicrobial potential of *C. aurantium* was investigated, and high

antimicrobial activity was recorded against *Bacillus subtilis* and *Staphylococcus aureus* with the minimum inhibition concentration (MIC) values of 2.7 mg/mL and 4.8 mg/mL. Moderate effects were detected against *Saccharomyces cerevisiae* and *Mucor ramannianus* with the values of 9.2 mg/ml and 5 mg/ml, respectively⁽¹⁵⁾.

- **Antioxidant activity**

In this study the antioxidant potentials of the peels and leaves of seven orange varieties obtained from Algeria were investigated by linoleic acid and β -carotene oxidation assays. The presence of phenolic compounds was also investigated. *C. aurantium* was found to have the highest phenol level. *C. aurantium* displayed the highest action on slowing down the rate of linoleic acid and β -carotene oxidation⁽¹⁶⁾. An antioxidant study was performed on the *C. aurantium* fruits on different ripening stages. According to the outcome obtained from DPPH free radical-scavenging and β -carotene/linoleic acid systems, the antioxidant activity was found to be varied related to the amount of the phenolic components⁽¹⁷⁾. *C. aurantium* peel and juice were suggested as a new potential source of natural antioxidants although, they were found to be less effective than butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and ascorbic acid, used as antioxidant standards. Anti-inflammatory activity of the flavonoid-type compounds of Korean *C. aurantium*, namely, nobiletin, naringin, and hesperidin was investigated. Inhibition of proinflammatory mediators by blocking nuclear factor-kappa B (NF-KB) and mitogen-activated protein kinase (MAPK) signaling in lipopolysaccharide- (LPS-) stimulated RAW 264.7 macrophages was assessed. The flavonoids were found to have the capacity to suppress the mRNA and protein expression of COX-2 and iNOS, by clarifying their anti-inflammatory action. A polymethoxy flavonoid-rich *C. aurantium* extract was shown to have a protective effect on alcohol-induced liver injury in an animal study via AMPK and Nrf2-related signal regulation⁽¹⁸⁾

- **Anti-ulcer Effects**

This study conducted to assess the effect of *C. aurantium* essential oil and its main compound limonene on gastric mucosa. The essential oil and its compound limonene were found to possess protective activity in the gastrointestinal system against lesions, which were induced by ethanol and nonsteroidal anti-inflammatory drugs in rats, at

doses of 250 mg/kg and 245 mg/kg, respectively. The essential oil and limonene increased the production of gastric mucus. The findings revealed that *C. aurantium* essential oil and its main compound limonene can be used as a promising target for the development of a novel gastro protective drug⁽¹⁹⁾.

In another study, the gastroprotective effect of β -myrcene, a monoterpene-type compound of *C. aurantium*, was evaluated. Experimental models of ulcer, induced by ethanol, NSAID stress, *Helicobacter pylori*, ischemia-reperfusion injury (I/R), and cysteamine (a drug used to treat cystinosis) was used to assess the ameliorative activity. β -Myrcene was administered at dose of 7.5 mg/kg. The results showed a potential role for β -myrcene against peptic ulcer disease. β -Myrcene contributed to the maintenance of integrity of the gastric mucosa with a significant decrease of ulcerative lesions, attenuating lipid peroxidative damage and preventing depletion of GSH, GR, and GPx⁽²⁰⁾

Citrus maxima

Common name: Pomelo, Bhogate, Shaddock, Papanus, Pummelo

● Antibacterial Activity

In this study the antibacterial activity of the fruit and leaf were examined. The fresh fruit samples were separated into peel, pulp and juice. Standard strain of *Staphylococcus aureus* (MTCC 3160), *Klebsiella pneumoniae* (MTCC 3384), *Pseudomonas aeruginosa*, (MTCC 424), *Salmonella typhi* (MTCC 3215), *Escherichia coli* (MTCC 40) were used for the study. Stock cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of colonies from the stock culture to peptone water and incubated for 4h at 37°C. Antibacterial activity was determined by agar disc diffusion method. Standard suspension of bacteria was inoculated on the surface of Muller-Hinton (Himedia) agar plates. Dimethyl Sulphoxide and Methanol (1:1) mixture was used to dissolve the plant extract. Sterilized filter paper discs (5mm) containing 20 μ L of each extract (100mg/mL) were arranged on the surface of the inoculated plates and incubated at 37°C for 18-24h. Gentamycin, Ciprofloxacin, Amikacin, Tetracycline, Streptomycin (10mg/disc (Himedia standard) was used as positive control and paper disc treated with DMSO was used as negative control. Each extract was analyzed in triplicate was

studied for antimicrobial activity. The extracts were found to have moderate activity against all organisms. Methanol extracts of leaves and pulp were found to have maximum activity compared to peel extracts against all microorganisms. Peel extract of *C. maxima* (red) has been shown to have the lowest activity (8-9mm) against all five microorganisms. Pulp extract of *C. maxima* (red) has registered moderate activity (12-8 mm) against all five microorganisms followed by the *C. maxima* (White)⁽²¹⁾

● Analgesic activity

This study investigates analgesic activity of crude methanolic extract of Peel of *C. maxima* fruits. Methanolic extracts of *C. maxima* peel with different concentration were tested for analgesic activity in mouse model of acetic acid induced writhing and formalin induced licking and biting.

In Acetic acid-induced writhing method the mice were divided into four groups each containing five mice. The analgesic activity of the samples was performed using acetic acid induced writhing model in mice. Test samples (300 and 500mg/kg body weight), vehicle (1% Tween 80 in water at the dose of 10 ml/kg p.o.) and Diclofenac sodium (10 mg/kg) were administered orally 30 min after the intra-peritoneal administration of 1% acetic acid. Then the mice were observed for specific contraction of the body referred to as 'writhing' for the next 20 min, complete writhing was not always accomplished by the animal, because sometimes the animals started to give writhing, but they did not complete it. This incomplete writhing was considered as half writhing. Accordingly, one full writhing was composed by two half-writhing. Diclofenac sodium (10 mg/kg) was used as a reference standard (positive control) and the number of writhes in each treated group was compared to that of a control group. About 87.13% inhibition of writhing was observed in mice treated with the reference drug; Diclofenac sodium (10 mg/kg). The methanol extract of peel of *C. maxima* fruits significantly reduced the acetic acid induced abdominal constrictions and stretching in a dose dependent manner compared to that of control. The analgesic effect of the extract at a dose of 300mg/kg was comparable to that of a dose 10 mg/kg of Diclofenac sodium⁽²²⁾

In Formalin induced licking and biting test the experimental animals (mice) were divided into four groups each containing five mice. 20 μ l of 2.5% formalin was injected into the dorsal surface of the

right hind paw 30 min before the administration of methanol extract of peel *C.maxima* fruits (300 and 500 mg/kg), vehicle (1% Tween 80 in water at the dose of 10 ml/ kg) and Diclofenac sodium (10 mg/kg).The crude extract at 300 and 500 mg/kg body weight showed a significant dose-response reduction in the hind paw licking compared to that of control. The extract at both tested doses showed better activity as compared to reference standard Diclofenac sodium at 10 mg/kg dose⁽²³⁾

- **Anti-inflammatory activity**

This study investigates anti-inflammatory activities of crude methanolic extract of Peel of *C.maxima* fruits. Methanolic extracts of *Citrus maxima* peel with different concentration were tested. Anti-inflammatory effect was tested by carrageenan induced paw edema model. The mice were divided into four groups each containing 5 mice. 0.1 ml of 1% carrageenan was injected into the plantar surface of the right hind paw to induce acute inflammation. The extract (300 and 500 mg/kg), normal saline (1 ml/kg) and Ibuprofen (10 mg/kg, i.p.) as the referral agents were administered 30 min after carrageenan injection. Vernier caliper was used to determine the diameter of edema by measuring the paw volume at 0, 1, 2, 3, and 4h during the study. The difference between the readings at time 1h and different time interval was taken as the thickness of edema. The extract exerted anti-inflammatory effect at the test dose of 300 and 500 mg/kg body weight which was comparable to that of the positive control group. The percent inhibition of carrageenan-induced inflammation at that doses were relatively low for initial 1hr period but had more pronounced effect subsequently at 2-3hr and was comparable to that of standard drug Ibuprofen at 10mg/kg dose. Moreover, it is notable that the dose independent effect of the extract or slightly better anti-inflammatory effect at the dose of 300mg/kg was found after 3-4hrs of extract administration⁽²³⁾

- **CNS depressant activity**

This study investigates CNS depressant activities of crude methanolic extract of Peel of *C.maxima* fruits. Methanolic extracts of *C.maxima* peel with different concentration were tested for the CNS depressant activity. The activity was evaluated by observing the reduction of locomotor activity by hole cross and open field test.

In Hole cross test a steel partition was fixed at the middle of a cage having a size of 30×20×14 cm. A hole of 3 cm diameter was made

at a height of 7.5 cm in the center of the cage. 20 mice were divided into four groups with five mice in each group. Mice of group-I received vehicle (1% Tween80 in water at the dose of 10 ml/kg p.o.), group-II received diazepam at 1mg/kg body weight (p.o.) while group-III and group-IV were treated with 300 and 500mg/kg body weight (p.o.) of the extract. The number of mice passing through the hole from one chamber to another was counted for a period of 3 min at 0, 30, 60, 90 and 120min after oral administration of test samples. The CNS depressant effect of the extract was instantaneous compared to the reference drug diazepam, since the number of movements at 0min was statistically significant but the number of movement of reference drug was not. Both doses (300 and 500mg/kg) of extract showed significant CNS depressant effect for the time of experiment tested and followed a dose dependent response. The obtained result revealed that the methanol extract of peel of *C.maxima* fruits was potent CNS depressant under the experimental conditions⁽²⁴⁾

In Open field test the animals were divided into control, standard and test groups. The control group received vehicle (1% Tween 80 in water at the dose of 10 ml/kg p.o.). The test group received the methanolic extract (at the doses of 300 and 500 mg/kg p.o.) and standard group received diazepam at the dose of 1mg/kg body weight orally. The animals were placed on the floor of an open field (100 cm×100 cm×40 cm h) divided into a series of squares. The number of squares visited by each animal was counted for 3hrs at 0,30, 60, 90 and 120min during the study period. The extract exhibited a decrease in the movements of the test animals at all dose levels. The results were statistically significant for all doses at 120 min and followed a dose-dependent response⁽²⁵⁾

- **Antioxidant activity**

In this study Anti-oxidant activity of *C.maxima* was carried out by using DPPH radical-scavenging activity of total phenol assay. Different concentrations of Soxhlet extract (0.03125, 0.0625, 0.125, 0.25, 0.5 and 1.0mg/ml) were prepared. The 1ml of different concentrations of extract solution and control (methanol, without extract) were added into individual centrifuge tubes. They were individually mixed with 5.0ml of methanolic solution of DPPH reagent. The mixtures were vortexed for few seconds and left to stand in the dark at room temperature for 30minutes. The absorbance was measured at 517nm against a blank by using UV-visible spectrophotometer. From this

research it may be concluded that *C.maxima* can exhibit antioxidant activity⁽²⁶⁾

● Antidiabetic activity

In this study the juice samples of *C.maxima* (Red and White) fruits were assessed for in vitro anti-diabetic activity by the α -amylase and α -glucosidase inhibition. In α -Amylase inhibition test the juice sample (100 μ L) was mixed with 100 μ L of 0.02 mol/L sodium phosphate buffer (pH 6.9) and 100 μ L α -amylase solution (4.5 units/mL/min) and pre-incubated at 25 °C for 10 min. Then, 100 μ L of 1% starch solution was added and incubated at 25°C for 30 min and the reaction was stopped by the addition of 1ml dinitrosalicylic acid reagent (1g 3,5-dinitrosalicylic acid in a solution containing 20 ml of 2 mol/L NaOH, 50 ml distilled water and 30g Rochelle salt. The contents were dissolved and the volume was made up to 100ml with distilled water). The test tubes were then incubated in a boiling water bath for 5min and then cooled to room temperature. The reaction mixture was then diluted 10-fold times with distilled water and the absorbance was measured at 540nm. In α -Glucosidase inhibition activity test the juice samples(100 μ L) were mixed with 100 μ L of 0.1 mol/L phosphate buffer (pH 6.9) and 100 μ L α -glucosidase solution (1 unit/mL/min) and incubated at 25°C for 5 min. After the pre-incubation, 100 μ L p-nitrophenyl- α -D-glucopyranoside (5 mmol/L) solution was added and the reaction mixture was incubated at 25°C for 10 min. After the incubation, the absorbance was recorded at 405nm. The potential inhibition of all juices against α -amylase ranged from 75.55% to 79.75%. The α -glucosidase inhibition activities of *C.maxima* (Red) and *C.maxima* (White) were 72.83%, and 71.88% respectively⁽²⁷⁾.

III. CONCLUSION

The fruits, flowers, leaves, essential oils and juices of citrus species are utilized to prepare various forms of food products. Along with their nutritional values, in the health industry, different parts of the plants of the citrus genus have been used as supplements or remedies to prevent or control diseases. This review focused on the pharmacological activities of *Citrus medica*, *Citrus aurantium* and *Citrus maxima*. The above mentioned citrus species produce secondary metabolites including flavonoids, alkaloids, limonoids, coumarins, carotenoids, phenolic acids and essential oils and their importance is due to their active properties. These characteristics

include anti-oxidant, anti-inflammatory, anti-cancer, antidiabetic, antimicrobial, analgesic, hypolipidemic, hypocholesterolemic, CNS depressant, antiulcer, antiobesity as well as cardiovascular protective effects etc.

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