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INVITRO EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *Citrus maxima*

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ABSTRACT

Hepatotoxicity, particularly drug-induced liver injury, remains a major clinical concern due to the liver's central role in metabolism and detoxification. The current work used the HepG2 cell line to assess the ethanolic leaf extract of *Citrus maxima* (Burm.) Merr.'s in vitro hepatoprotective efficacy against paracetamol-induced cytotoxicity. *C. maxima* leaves collected, checked, shade-dried, and then extracted by maceration with 70% ethanol. Preliminary phytochemical screening was done, and the extract's percentage yield was calculated. The MTT test was used to measure hepatoprotective activity at doses between 6.25 and 100 µg/mL. Flavonoids, phenolic substances, tannins, alkaloids, and terpenoids were found by phytochemical examination. Hepatotoxicity was confirmed by the significant reduction in cell viability following paracetamol therapy. Cell viability increased in a concentration-dependent manner after administration with the ethanolic extract of *C. maxima*; higher concentrations (25–100 µg/mL) demonstrated significant protection against damage caused by paracetamol. It was discovered that the extract successfully restored cell viability and was not harmful to normal cells. The extract's phytoconstituents' cytoprotective and antioxidant properties may be responsible for the hepatoprotective effect that was seen. These results indicate *Citrus maxima*'s potential as a natural hepatoprotective agent and validate its historic use in liver problems. To validate these findings, more in vivo and molecular research is necessary.

KEY WORDS

Hepatotoxicity, *Citrus maxima,* Pharmacological activities, MTT Assay

INTRODUCTION

Liver is the fundamental organ for digestion and metabolism of medicines.[1] The liver is the body's largest gland and the second largest organ, weighing approximately 1.4 kg. It has a reddish-brown hue and a rubbery texture. The liver is vital for numerous physiological functions, including metabolism, immune regulation, detoxification, digestion, and the storage of vitamins.[2] The causes of liver disorders are influenced by factors like nutritional deficiencies, biochemical imbalances, bacterial or viral infections, and environmental disruptions.[3] The smooth endoplasmic reticulum of the liver serves as the primary metabolic processing centre for both naturally occurring substances such as cholesterol, steroid hormones, fatty acids, and proteins and foreign compounds like drugs and alcohol. Due to its crucial role in detoxifying and transforming these chemicals, the liver is particularly vulnerable to toxic damage.[4]

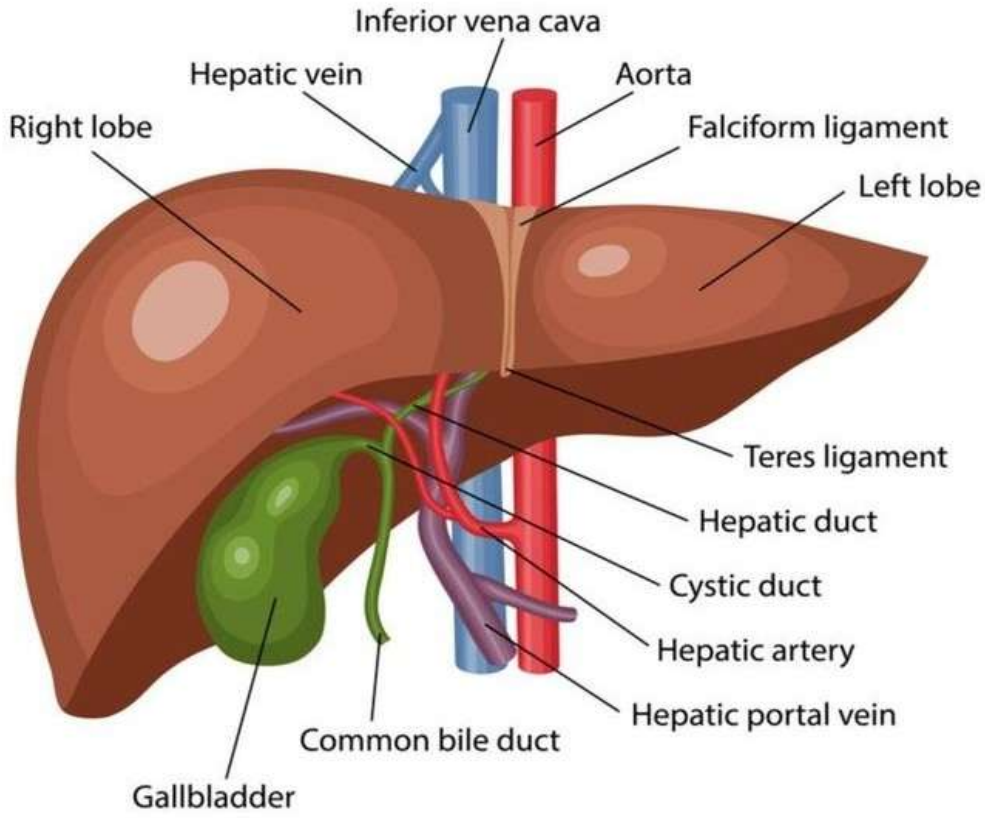

Figure 1.1: Structure of Liver

Hepatotoxicity refers to impaired liver function or liver injury resulting from excessive exposure to drugs or foreign chemical substances (xenobiotics). [8] Substances that cause liver damage are known as hepatotoxins or hepatotoxicants**.** Hepatotoxicants are externally derived substances with clinical significance, which can include excessive doses of specific medications, industrial chemicals, naturally occurring toxins such as microcystins, as well as certain herbal remedies and dietary supplements.[9] Some medications can lead to liver damage even when administered within their prescribed therapeutic limits. Hepatotoxicity may arise not only from the direct toxicity of the drug itself but also from reactive metabolites or immune-mediated responses that impact hepatocytes, bile duct cells, and/or the liver's vascular system. [10] The concentration of the toxicant, which can be either a parent compound or a toxic metabolite, the differential expression of enzymes, and the gradient of cofactor concentrations in blood throughout the acinus all influence the hepatotoxic response that a chemical agent elicits. [11]

Symptoms associated with hepatotoxicity can include jaundice or icterus, characterized by yellowing of the skin, eyes, and mucous membranes due to elevated bilirubin levels in the extracellular fluid. Other signs may involve pruritus (itching), intense abdominal pain, nausea or vomiting, weakness, extreme fatigue, persistent bleeding, skin rashes, widespread itching, swelling in the feet and/or legs, sudden and unusual weight gain, dark-coloured urine, and pale stools.[12] Drug-induced liver injury (DILI) represents a major type

of hepatotoxicity and plays a significant role in cases of acute liver failure, especially in industrialized countries.[13]

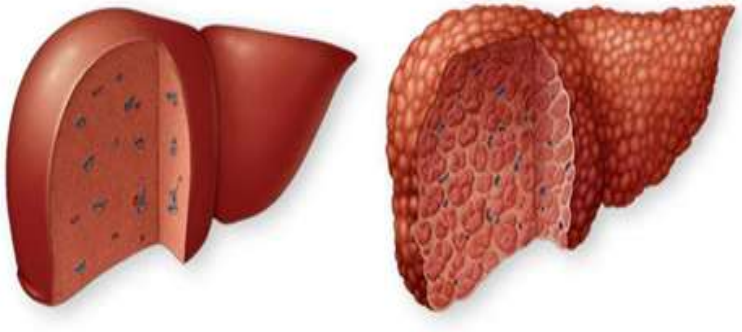

Figure 1.2: Structure of Hepatotoxic Liver

Citrus maxima (Burm). Merr. belongs to the family Rutaceae. It is a perennial tree that is frequently referred to as Pomelo, Bhogate, Shaddock, Papanus, Pummelo, etc. in different parts of the world. The plant is indigenous to Asia and is commercially propagated in China, Nepal, Thailand, Malaysia, India, Vietnam, Indonesia, Philippines, Japan, and many other Asian nations. Lately, it has been introduced to numerous tropical nations. [14-16]

C. maxima, which include the entire plant, whole fruit, albedo, and pulp. Its large, spherical, delicious fruits have either white or pink flesh. Its fruit has a diameter of 12–18 cm, while the fruit wall is 2–2.5 cm thick. A green exocarp known as flavedo is the oil-rich component with a characteristic aroma and is frequently exploited in the extraction of essential oils. Depending on the variety, albedo, a white, spongy mesocarp, is around 2 cm thick. [17] It is traditionally used for ulcers, febrifuge, dyspepsia, lumbago, fever, cardiotonic, gastrointestinal conditions, diabetes, and cardiovascular disease. [18-23]

Figure 3.1: *Citrus maxima* plant

BOTANICAL DESCRIPTION

The leaves are arranged in a cauline insertion with an alternate pattern, simple in design, shaped like ovate-lanceolate, petiolate with a crenate edge, having an emarginated tip, symmetrical base, and slick, shiny surfaces. The upper side was green, darker than the underside, displaying pinnate reticulate venation. The midrib was more pronounced on the underside, with the lamina dimensions measuring between 5-20 cm in length and 2-12 cm in width. The petiole was winged, green in hue, measuring 2-4 cm in length and 2-2.5 cm in diameter.

The fruit ranges from nearly round to oblate or pear shaped; 10-30 cm wide; the peel, adhering, readily removed, greenish yellow or pale yellow, minutely hairy, sprinkled with tiny green glands. The pomelo peel, which can make up as much as 30% of the weight of the fresh fruit, is the biggest and thickest of all citrus fruits. Pulp it is separated into 11 to 18 segments, varies in colour from greenish yellow or pale yellow to pink or crimson, and can be extremely juicy or somewhat dry. The sacs may cling to one another or be loosely connected, and the segments are easily peeled.

The pulp's flavour ranges from bland and somewhat sweet to subacid or strongly acidic, perhaps with a tinge of bitterness.

The fruit's albedo is white and has a spongy texture, while the fruit's rind is green with oil glands visible as dots throughout the fruit peel. The lamella, a rough skin, covers the fruit's segments. Large, yellowish white seeds with a white interior are rare, yet certain fruits may have a lot of seeds [24].

CHEMICAL CONSTITUENTS

Phytochemicals belonging to different chemical classes such as alkaloids, saponins, carbohydrates, phenols, flavonoids, glycosides, anthraquinone, amino acids, carotenoids, and terpenoids are present. [25-28]

SL NO	PLANT PART/EXTRACT	CONSTITUENTS
1.	Leaves	Geraniol, Linalool, Limonene, Acacetin, Cosmosiin, Luteolin, Narirutin, Neohesperidin, Limonin
2.	Flower	Geranyl formate, Geranyl aceatate, (z)-Ocimene
3.	Fruit	Isosinensetin, Narirutin, Neohesperidin, Nobiletin
4.	Peel	Ferulic acid, Gallic acid, Vanillic acid, β-Sitosterol, Deoxylimonin, Limonin
5.	Seed	Caffeic acid, Deoxylimonin, Limonin, Nomilinic acid

PHARMACOLOGICAL ACTIVITIES

1. Antioxidant Activity

The extract of *Citrus maxima* have been found to posses antioxidant properties which can help to protect against oxidative stress and cell damage.[29]

2. Anti-inflammatory Activity

The plant's extracts have been shown to exhibit anti-inflammatory activity, which may help to reduce inflammation.[30]

3. Antimicrobial Activity

The extract of *Citrus maxima* have been found to exhibit antimicrobial activity against various bacteria and fungi, which help prevent or treat infections.[31]

4. Analgesic Activity

The extract of *Citrus maxima* have been found to possess analgesic activity.[30]

5. Anticancer Activity

Studies have shown that *Citrus maxima* extracts exhibit anticancer activity against various cancer cell lines, which may help prevent or treat cancer.[32]

6. Hepatoprotective Activity

The plant's extracts have been found to posses hepatoprotective properties, which may help to treat liver damage.[33]

MEDICINAL USES

Antioxidant, antibacterial, anti-inflammatory, analgesic, anticancer, antidiabetic, anti-Alzheimer's disease, insecticidal, anxiolytic, hepatoprotective, antimalarial, and antiobesity are only a few of the plant's notable bioactivities.

MATERIALS AND METHODS

Collection and authentication of plant material:

The plant *Citrus maxima* were collected from Parassala, Thiruvananthapuram and leaves of the same plant was collected for the study purpose and was given for authentication. The plant was identified and authenticated from Dr Lubaina A S professor and head of department of botany, Christain college, Kattakada. The sample was collected and washed and dried under shade for a week. The dried leaves grinded using grinder and made into coarse powder. This coarse powder is stored in air tight container.

Preparation of ethanolic leaves extract of *Citrus maxima*

Leaves of *Citrus maxima* were washed under running tap water and dried under shade for 15 days and pulverized into coarse powder by the aid of mechanical grinder. For the preparation of the ethanolic extract 100g of pulverized powder is extracted by maceration technique with 500ml of 70% ethanol. The mixture is subjected to continuous shaking at room temperature and kept closed with aluminium foil in a dark area. After 1 week the extract is strained with muslin cloth and the crude extract was concentrated to dryness under reduced pressure and controlled temperature and stored in airtight container for further use.

Calculation of Percentage yield

The percentage yield of extract was calculated using the formula:

$$\textbf{Percentage Yield} = \frac{W2}{W1} \times 100$$

Where,

W1– Weight in grams of dried plant material

W2 – Weight in grams of extract obtained

Preliminary Phytochemical Screening

Qualitative phytochemical screening was carried out for flavonoids, tannins, phenolic compounds, alkaloids, saponins and terpenoids.

INVITRO STUDY OF HEPATOPROTECTIVE ACTIVITY

MTT Assay [34,35]

Procedure

The cells (10,000 cells/well) were seeded on 96 well plates and allowed to acclimatize to the culture conditions such as 37°C and 5% CO2 environment in the incubator for 24 hours. The samples (10mg/mL) were prepared in DMEM media and added to the wells containing cultured cells at final concentrations of 6.25, 12.5, 25, 50 and 100 µg/mL respectively. Untreated wells were kept as control for 24h. Paracetamol (300µg/mL) was added to test compound pre-treated cells and paracetamol alone treated cells were kept as positive control and

incubated for 24h. All the experiments were done in triplicate and average values were taken in order to minimize errors. After incubation period, the media from the wells were aspirated and discarded. 100 µL of 0.5 mg/mL MTT solution in PBS was added to the wells. The plates were further incubated for 2 hours for the development of formazan crystals. The supernatant was removed and 100 µL DMSO (100%) were added per well.

The absorbance at 570 nm was measured with micro plate reader. Three wells per plate without cells served as blank.

All the experiments were done in triplicates.

The cell viability was expressed using the following formula:

$$Percentage\ of\ cell\ viability = \frac{\text{Average absorbance of treated}}{\text{Average absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

Percentage yield

Table No 1: Percentage yield of plant extract of *Citrus maxima*

NAME OF PLANT	PLANT PART USED	METHOD OF EXTRACTION	SOLVENT USED FOR EXTRACTION	PERCENTAGE YIELD (%w/w)
Citrus maxima	Leaf	Maceration	Ethanol	7.020 %w/w

Preliminary phytochemical screening

Table No 2: Preliminary phytochemical screening of ethanolic leaf extract of *Citrus maxima*

SL.NO.	NAME OF THE CONSTITUENTS	NAME OF THE TEST	INFERENCE
1.	Flavonoids	Alkali test	Positive
		Lead acetate test	Positive
2.	Tannins	Ferric chloride test	Positive
3.	Phenolic compounds	Lead acetate test	Positive
4.	Alkaloids	Hager's test	Positive
		Dragendorff's test	Positive
5.	Saponins	Foam test	Negative
6.	Terpenoids	Salkowski test	Positive

Invitro study of *Citrus maxima*

MTT (3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyltetrazolium bromide) Assay:

At increasing concentrations, ethanolic extract of *Citrus maxima* shows decreasing cytotoxicity in HepG2 cells administered with different concentrations of the sample. Thus the sample studied non-cytotoxic to normal cell line. The sample was found to be non-cytotoxic to normal cell lines.

Effect of ethanolic extract of *Citrus maxima* in MTT Assay

SAMPLE CODE:S

Table 3: MTT assay results for varying concentration of test sample

Concentration (µg/ml)	Triplicate 1	Triplicate 2	Triplicate 3	Average OD
Control	0.510	0.505	0.495	0.503
Paracetamol	0.220	0.230	0.215	0.222
6.25	0.245	0.255	0.250	0.250
12.5	0.290	0.310	0.300	0.300
25	0.355	0.365	0.360	0.360
50	0.420	0.435	0.425	0.427
100	0.470	0.485	0.480	0.478

Table 4: Percentage of viability for varying concentration of test sample

Concentration (µg/ml)	Percentage of viability
Paracetamol	40.13
6.25	49.67
12.5	59.60
25	71.52
50	84.77
100	90.00

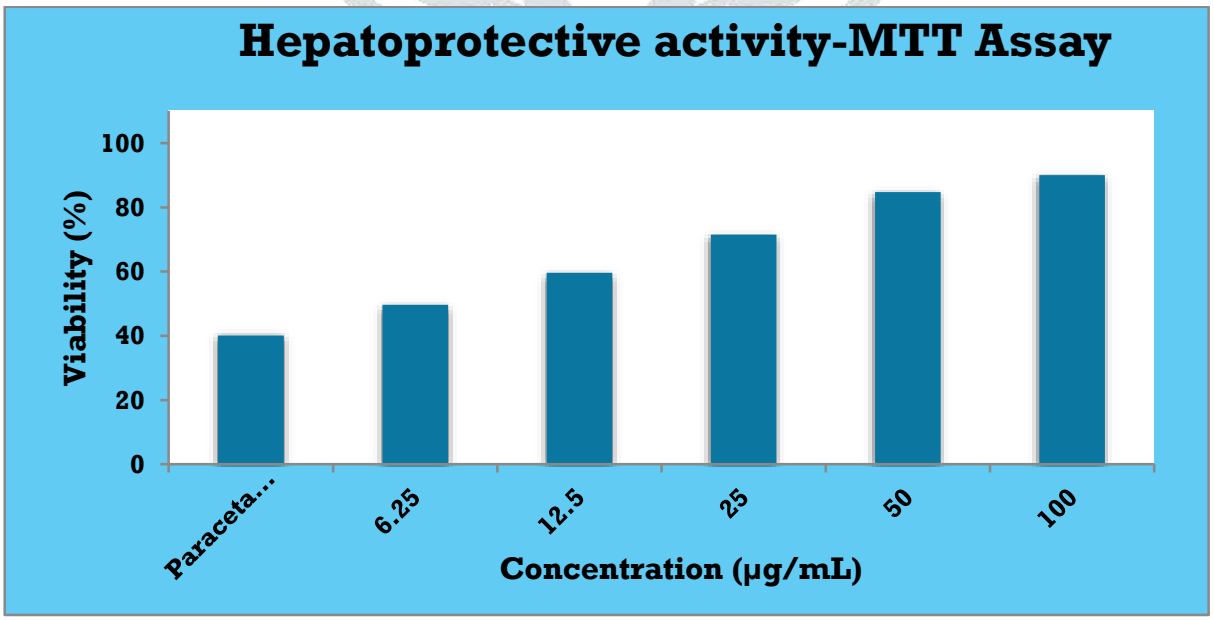

Figure 5: Graphical representation of MTT assay

DISCUSSION

The liver is very susceptible to damage from xenobiotics like medicines and chemicals since it is essential to metabolism, detoxification, and the preservation of physiological homeostasis. Because excessive metabolism produces reactive intermediates that lead to oxidative stress, mitochondrial malfunction, and hepatocyte mortality, paracetamol-induced hepatotoxicity is a well-established experimental model. Plant-based treatments with antioxidant and cytoprotective capabilities are becoming more popular due to the drawbacks and side effects of synthetic hepatoprotective drugs. In this context, the present studies used the HepG2 cell line model to assess the ethanolic leaf extract of *Citrus maxima*'s hepatoprotective efficacy in vitro.

Citrus maxima (Burm.) Merr., a perennial medicinal plant of the Rutaceae family, has been traditionally used to treat liver problems, inflammation, gastrointestinal issues, and metabolic diseases. The plant is well-known for its rich medicinal value and is found throughout Asia, including India. Antioxidant, anti-inflammatory, antibacterial, and hepatoprotective properties have been demonstrated in a number of *C. maxima* parts, particularly the leaves and fruit peel. The long history of traditional use and the presence of bioactive secondary metabolites support its selection for the current study's assessment of hepatoprotective potential.

In this study, polar and moderately polar phytoconstituents are obtained from *Citrus maxima* leaves by maceration with 70% ethanol. Flavonoids, phenolic chemicals, tannins, alkaloids, and terpenoids were found in the ethanolic leaf extract after a preliminary phytochemical screening; saponins were not found. Hepatocytes protected from oxidative damage and toxic damage by these phytoconstituents, especially flavonoids and phenolics, which are well known for their antioxidant, free radical-scavenging, and membrane-stabilizing properties.

HepG2 cells exposed to paracetamol-induced toxicity were used in the MTT test to assess the ethanolic leaf extract's hepatoprotective activity. Because an excess of paracetamol causes oxidative stress and mitochondrial malfunction, which lowers cell viability, it was used as a hepatotoxic agent. Significant hepatic toxicity was confirmed by the current results, which showed that paracetamol therapy significantly reduced HepG2 cell viability to almost 40%. Cell viability improved in a dose-dependent manner after treatment with *C. maxima* extract; cell viability increased in a concentration-dependent manner following treatment with *C.maxima* extract. Higher dosages (25-100 µg/ml) demonstrated a significant restoration of cell viability, approaching that of the untreated control, whereas lower concentrations (6.25 and 12.5 µg/ml) indicated moderate protection. These results show that the extract effectively protects hepatocytes from damage caused by paracetamol, most likely via cytoprotective and antioxidant processes mediated by flavonoids and phenolic substances. Overall, the results justify the traditional use of *Citrus maxima* leaves in liver diseases and support its hepatoprotective potential.

CONCLUSION

The results of the present study indicate that *C.maxima* leaf extract exhibits promising hepatoprotective activity in HepG2 cells against paracetamol-induced cytotoxicity. The observed protective effect may be broadly attributed to the presence of phenolic compounds and flavonoids reported in *C.maxima,* which are known to contribute to antioxidant and cytoprotective properties. These phytoconstituents may play a role in reducing oxidative stress and improving cellular viability, thereby supporting hepatocyte survival. Although the exact mechanisms were not investigated in the present study, the findings provide preliminary evidence supporting the traditional use of *C.maxima* in liver-related disorders. Further studies involving detailed phytochemical characterization, mechanistic evaluation, and in vivo models are required to substantiate these observations.

REFERENCE

1. Singh A, Bhat TK, Sharma OP. Clinical biochemistry of hepatotoxicity. J Clin Toxicol. 2011; 4:1–19.
2. Saxena R, Theise ND, Crawford JM. Microanatomy of the human liver: exploring the hidden interfaces. Hepatology. 1999;30(6):1339–46.
3. Fernandez-Checa JC, Hirano T, Tsukamoto H, Kaplowitz N. Mitochondrial glutathione depletion in alcoholic liver disease. Alcohol. 1993; 10:469–75.
4. Saukkonen JJ, Cohn DL, Jasmer RM, Schenker S, Jereb JA, et al. An official ATS statement: hepatotoxicity of antituberculosis therapy. Am J Respir Crit Care Med. 2006; 174:935–52.
5. Almazroo OA, Miah MK, Venkataramanan R. Drug metabolism in the liver. Clin Liver Dis. 2017;21(1):1–20.
6. O'Brien L, Hosick PA, John K, Stec DE, Hinds TD. Biliverdin reductase isozymes in metabolism. Trends Endocrinol Metab. 2015 Apr;26(4):212–20.
7. Hoekstra LT, de Graaf W, Nibourg GA, Heger M, Bennink RJ, Stieger B, et al. Physiological and biochemical basis of clinical liver function tests: a review. Ann Surg. 2013 Jan;257(1):2736.
8. Navarro VJ, Senior JR (2006) Drug – related hepatotoxicity. N Engl J Med 354: 731-739
9. Willett KL, Roth RA, Walker L (2004) Workshop overview: Hepatotoxicity assessment for botanical dietary supplements. Toxicol Sci 79: 4-9
10. Deng X, Luyendyk JP, Ganey PE, Roth RA. Inflammatory stress and idiosyncratic hepatotoxicity: hints from animal models. Pharmacol Rev. 2009; 61:262–82.
11. Kedderis GL. Biochemical basis of hepatocellular injury. Toxicol Pathol. 1996; 24:77–83.
12. Bleibel W, Kim S, D'Silva K, Lemmer ER. Drug-induced liver injury: review article. Dig Dis Sci. 2007; 52:2463–71.
13. Reuben A, Koch DG, Lee WM. Drug-induced acute liver failure: results of a U.S. multicenter, prospective study. Hepatology. 2010;52(6):2065–76.
14. Louzada ES, Ramadugu C. Grapefruit: history, use, and breeding. **HortTechnology**. 2021;31:1–11.
15. Susandarini R, Subandiyah S, Rugayah R, Daryono BS, Nugroho LH. Assessment of taxonomic affinity of Indonesian pummelo (*Citrus maxima* (Burm.) Merr.) based on morphological characters. **Am J Agric Biol Sci**. 2013;8:182–190.
16. Lim TK. *Citrus maxima*. In: **Edible Medicinal and Non-Medicinal Plants**. Berlin: Springer Science and Business Media; 2012. p. 151–167.
17. Thielen M, Speck T, Seidel R. Viscoelasticity and compaction behaviour of the foam-like pomelo (*Citrus maxima*) peel. **J Mater Sci**. 2013;48:3469–3478.
18. Singh A. *Citrus maxima* (Burm.) Merr., a traditional medicine: its antimicrobial potential and pharmacological update for commercial exploitation in herbal drugs—a review. **Int J ChemTech Res**. 2017;10:1–10.
19. Singh A, Navneet. Evaluation of antimicrobial potential and phytochemical assessment of *Citrus maxima* Burm. seed extracts against respiratory tract pathogens. **N Y Sci J**. 2016;9:1–7.
20. Cheong MW, Liu SQ, Zhou W, Curran P, Yu B. Chemical composition and sensory profile of pomelo (*Citrus grandis* (L.) Osbeck) juice. **Food Chem**. 2012;135:2505–2518.
21. Kharjul A, Mangesh K, Vilegave K, Chandankar P, Gadiya M. Pharmacognostic investigation on leaves of *Citrus maxima* (Burm.) Merr. (Rutaceae). **Int J Pharm Sci Res**. 2012;3(12):4913–4918.
22. Buachan P, Chularojmontri L, Wattanapitayakul SK. Selected activities of *Citrus maxima* Merr. fruits on human endothelial cells: enhancing cell migration and delaying cellular aging. **Nutrients**. 2014;6(4):1618–1634.
23. Gautam IP, Gotame TP. Diversity of native and exotic fruit genetic resources in Nepal. **J Nepal Agric Res Counc**. 2020;6:44–55.
24. Sawant T, Panhekar D. A brief review on recent advances of *Citrus maxima* (Chakota). **Int J Recent Sci Res**. 2017;8(8):19400–19416.
25. Ani PN, Abel HC. Nutrient, phytochemical, and antinutrient composition of *Citrus maxima* fruit juice and peel extract. **Food Sci Nutr**. 2018;6(3):653–658.
26. Athira U. Evaluation of carbohydrate and phenol content of citrus fruit species. **Int J Appl Res Nat Prod**. 2017;3(9):160–164.
27. Abirami A, Nagarani G, Siddhuraju P. Hepatoprotective effect of leaf extracts from *Citrus hystrix* and *Citrus maxima* against paracetamol-induced liver injury in rats. **Food Sci Hum Wellness**. 2015;4(1):35–41.

28. Sapkota B, Jain V. Evaluation of anti-ulcer activity of *Citrus maxima* (Burm.) leaf extract in experimental animals. **J Clin Exp Pharmacol**. 2021;11(2):1–6.
29. Oyedepo TA. Antioxidant potential of *Citrus maxima* fruit juice in rats. **Glob Adv Res J Med Med Sci**. 2012;1(5):122–126.
30. Ibrahim M, Amin MN, Millat S, Raju JA, Hussain S, Sultana F, Islam MM, Hasan MM. Methanolic extract of peel of *Citrus maxima* fruits exhibits analgesic, CNS depressant and anti-inflammatory activities in Swiss albino mice. **BEMS Rep**. 2018;2(2):64–70.
31. Yathiender S. A comparative study of antimicrobial activity of *Citrus maxima* and *Citrus aurantium* plant extracts. **Int J Recent Sci Res**. 2017;8(7):18507–18509.
32. Kundusen S. Antitumor activity of *Citrus maxima* (Burm.) Merr. leaves in Ehrlich's ascites carcinoma cell-treated mice. **ISRN Pharmacol**. 2011;2011:Article ID 138737.
33. Abirami A, Nagarani G, Siddhuraju P. Hepatoprotective effect of leaf extracts from *Citrus hystrix* and *Citrus maxima* against paracetamol-induced liver injury in rats. **Food Sci Hum Wellness**. 2015;4(1):35–41.
34. Mosmann, T. (1983) Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. Journal of Immunological Methods, 65, 55-63.
35. Joseph, M.M., Aravind, S.R., Varghese, S., Mini, S. and Sreelekha, T.T., 2012. Evaluation of antioxidant, antitumor and immunomodulatory properties of polysaccharide isolated from fruit rind of Punicagranatum. Molecular medicine reports, 5(2), pp.489-496.