IJPSR (2024), Volume 15, Issue 6 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 December 2023; received in revised form, 25 May 2024; accepted, 28 May 2024; published 01 June 2024

IN-VITRO EVALUATION OF ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF *AEGLE MARMELOS, LAWSONIA INNERMIS AND MURRAYA KOENGII*

Srikar Barik [*1] and P. K. Panda [2]

Department of Pharmacology [1], UDPS, Utkal University, Vanivihar, Bhubaneswar - 751004, Odisha, India.

Akash Institute of Pharmaceutical Education & Technology [2], Mahipal, Kendrapara - 754211, Odisha. India.

Keywords:

Aegles marmelos, *Lawsonia innermis*, *Murraya koenigii*, *In-vitro* evaluation, Hepatoprotective

Correspondence to Author:
Srikar Barik

Ph.D. Research Scholar,
Department of Pharmacology,
UDPS, Utkal University, Vanivihar,
Bhubaneswar - 751004, Odisha, India.

E-mail: srikar.barik@gmail.com

ABSTRACT: The present study was conducted with an objective to evaluate the *in-vitro* antioxidant and hepatoprotective activity of three different medicinal plants. Due to the adverse effects of therapeutic agent with better hepatoprotective activity in modern medicine several plants have been screened for the aforesaid activity during last few decades detailed literature survey revealed that leaves of *Aegles marmelos*, *Lawsonia innermis* and *Murraya koenigii* plants were traditionally used for their liver protective activity. When healthcare providers refer to liver disease, they're usually referring to chronic conditions that do progressive damage to your liver over time. Chronic liver diseases are not uncommon worldwide and are characterized by progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma. There is growing evidence that free radicals and reactive oxygen species play an important role in the progression of liver diseases and thus plants with free radical scavenging activity could play a crucial role in preventing the progression of liver disorders. The results of the present study demonstrate that, all 3 plants which were selected based on their traditional and ethnomedical claim possess antioxidant activity, polyphenolic profiling and potential hepatoprotective efficacy. As a result, the study's significant findings demonstrate that it is a step towards evidence-based phytomedicine.

INTRODUCTION: The liver is a major organ that plays an important role in the metabolism and elimination of xenobiotics from the human body. The liver is responsible for numerous vital tasks in our survival, including blood purification, detoxification, cholesterol control, digestion, and storage.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.15(6).1859-69

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.15(6).1859-69

It is involved in practically all metabolic pathways leading to growth, disease resistance, nutrition delivery, energy provision, and reproduction [1-3].

Hepatic disease or Liver disease is a term that affects the cells, tissues, various structures, or functions of the liver while Liver cell injury is generally caused by various toxic chemicals (certain anti-biotic, chemotherapeutic agents, carbon tetrachloride (CCl4), thioacetamide (TAA) *etc.*), excessive alcohol consumption and microbes is well stdudied [4-7]. The available synthetic drugs in the market to treat liver diseases in this condition also increase damage to the liver. Hence, herbal medicines have been used in the treatment of liver

diseases for a long time, so the maintenance of a healthy liver is possible [8-11].

***Aegle marmelos*:** Commonly known as Bael in Bangla, is a tree belonging to the Rutaceae family that grows in various regions of Bangladesh, India, and Southeast Asia. Numerous bioactive compounds, including cinnamic acid, aegeline, skimmianine, lupeol, cineole, citral, citronella, cuminaldehyde, eugenol, marmesinin, marmelosin, luvangetin, aurapten, psoralen, marmelide, fagarine, marmin and tannin, have been isolated from the plant. Previous studies have reported that different parts of *A. marmelos* have therapeutic uses, such as treating asthma, anaemia, fractures, wound healing, swollen joints, high blood pressure, jaundice, diarrhoea, intermittent fever, intestinal ailments, fertility control, treatment after childbirth, and fish poison [12-15]. *A. marmelos* is also a traditional herbal medicine for treating diabetes mellitus in folk systems of medicine in India, Bangladesh, and Sri Lanka. The unripe dried fruit is used to cure diarrhoea and dysentery due to its astringent, digestive, and stomachic properties. A sweet drink prepared from the fruit pulp provides a soothing effect on patients who have just recovered from bacillary dysentery [16-20]. Given the medicinal value of various parts of *A. marmelos*, this study aims to investigate the antioxidative and cytotoxic properties of three different fractions of *A. marmelos* leaves to identify potential new therapeutic uses.

***Lawsonia inermis*:** Commonly known as henna, is a perennial tree belonging to the Lythraceae family. It thrives in arid and warm regions. Henna is cultivated as an ornamental and commercial dye crop and is found predominantly in tropical, sub-tropical and semi-arid areas of Africa, south Asia, and north Australia. Extensive research has revealed a wide range of chemical constituents present in Henna, including naphthoquinone derivatives, phenolic derivatives, coumarins, xanthones, tannins, flavonoids, aliphatic components, triterpenes, sterols, glucose, gallic acid, amino acids, mannitol, trace elements, and minerals. Henna has been traditionally used in folk medicine for its medicinal [23]. It has been employed as an astringent, hypotensive, sedative, and treatment for ailments such as headaches, jaundice, leprosy, skin diseases, venereal diseases, smallpox, and spermatorrhea. The bark of the Henna tree has been utilized in the treatment of burns, jaundice, spleen enlargement, calculus, leprosy, and skin disorders. The root of the tree has been considered a potent medicine for gonorrhoea, herpes infection, sore eyes, as an abortifacient, and for the treatment of certain nervous disorders. Additionally, the seeds of Henna have shown potential in alleviating liver disorders, prompting further investigation into the protective effects of other parts of the plant [24-28].

***Murraya koenigii*:** A member of the Rutaceae family, is commonly referred to as curry leaf or curry veppila in various Indian dialects. This small tree or shrub, which can reach heights of 4-6m, emits a strong aroma and provides ample shade. Native to tropical Asia, *Murraya koenigii* is found throughout India and other parts of the world. In South India, the leaves of this plant are used as a natural flavouring agent in a variety of curries. Additionally, these leaves have been traditionally used in the Indian Ayurvedic system to treat diabetes. The plant possesses acrid, analgesic, bitter, cooling, alexiteric, anthelmintic, carminative, purgative, and stimulant properties, and is used to alleviate body heat, blood disorders, diarrhoea, dysentery, eruptions, inflammation, itching, kidney pain, leukoderma, piles, snakebite, thirst, and vomiting. In traditional medicine, it is employed as an antiemetic, antidiarrheal, dysentery, febrifuge, blood purifier, tonic, stomachic, and flavouring agent in curries and chutneys [29-32]. The use of plant-based medicine has long been a cornerstone of traditional societies in addressing health issues, as plants contain biologically active compounds known as phytoconstituents [33-35].

METHODOLOGY:

Collection of Plant Material: The leaves of *Aegle marmelos*, *Lawsonia inermis*, *Murraya koenigii* were collected from the Rajgangpur, Sundargarh, Odisha, India during the months of September-December 2021 and authenticated by Professor Archana Satpathy, Department of Botany, Govt. Autonomous College, Rourkela. The voucher specimens (Rutaceae/2021/1), Lythraceae/2021/2, Rutaceae/2021/3 have been kept in the herbarium for future reference.

Preparation of Plant Extracts: Successive extracts of the selected medicinal plants were prepared by extracting successively with petroleum ether (60-80°), chloroform, acetone, methanol and water in Soxhlet apparatus. All the three plants have showed higher % yield in methanol and aqueous showing the presence of a greater number of polar compounds followed by less polar (chloroform) and lastly non-polar (petroleum ether). Acetone which is a ketone class having the same polarity as of methanol showed less percentage yield in all the selected plants.

***In-vitro* Antioxidant Studies:**

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay: DPPH free radical scavenging activity of different extracts of leaf of *Aegles marmelos, Lowsonia innermis* and *Murraya koenigii* plants were measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) DPPH solution (0.1 mM) was prepared in methanol and 1 ml of this solution was added to 1 ml extract solution in methanol at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. Absorbance was measured at 517 nm in a spectrophotometer against blank containing 1 ml of methanol and 1 ml 0.1 mM DPPH solution. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Ascorbic acid was used as a standard antioxidant compound. Percentage scavenging was calculated by using the following formula and IC50 values were reported [36, 37].

% scavenging = Abs of control-Abs of sample / Abs of control × 100

Where, Abs Control = Absorbance of DPPH + Methanol, Abs Sample = Absorbance of DPPH + Standard/ plant extract.

ABTS (2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) Radical Scavenging Assay: ABTS radical was generated by mixing 7 mM ABTS solution and 2.4 mM potassium persulfate solution in equal quantity. This solution was allowed to react at room temperature in dark for 12 hours. The solution was then diluted with methanol to obtain an absorbance of 0.7 to 0.8 units at 734 nm. Fresh ABTS radical solution was prepared for each assay. For the assay purpose 1 ml of plant extract at different concentrations were allowed to react for 7 min with 1 ml of the ABTS radical solution as prepared above and the absorbance was measured at 734 nm using the spectrophotometer against blank containing 1 ml methanol and 1 ml ABTS radical solution. Percentage scavenging was calculated as per the formula mentioned in DPPH radical scavenging assay. Trolox was used as standard antioxidant compound [38, 39].

O-phenanthroline Assay: 1, l0-Phenanthroline-iron (III) reagent was prepared by mixing 0.198 g of l, l0-phenanthroline monohydrate, 2 ml of 1M hydrochloric acid and 0.16 g of ferric ammonium sulphate and diluting with water to 100 ml. For antioxidant testing 0.2 ml standard or extract (different concentrations) was mixed with 0.2 ml 1, l0-Phenanthroline-iron (III) reagent, 0.6 ml methanol and 4 ml water. The solution was incubated at 50 °C for 30 min and absorbance was read at 510 nm against reagent blank (i.e., without standard/extract). Ascorbic acid was used as standard [40, 41]. Percentage scavenging was calculated by using the following formula.

% scavenging = Abs of control-Abs of sample / Abs of control × 100

Total Antioxidant Capacity: Total antioxidant capacities of different extracts of leaves of *Aegles marmelos*, *Lowsonia innermis* and *Murraya koenigii* plants were determined according to the method explained by Pan *et al.* (2008) and Prieto *et al.* (1999). The extract sample solutions (0.1ml, 1000 µg/ml) were mixed with 0.3 ml of reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate.

The reaction mixture was incubated in a water bath at 95 °C for 90 minutes allowed to cool to room temperature and the absorbance was measured at 695 nm against a blank. The blank solution contained all the reagents except the test sample. Trolox was used as standard. The antioxidant activity was expressed as mg/g Trolox equivalent. The assay is based on the reduction of molybdate (VI) to molybdate (V) by extracts and subsequent formation of a green phosphate/ Mo (V) complex at acid ph. The high absorbance values indicated that the sample possesses significant antioxidant activity [42, 43].

***In-vitro* Cytotoxicity Studies For Selected Plant Extracts by MTT Assay:**

Preparation of Test Solutions: 10 mg of the extract samples were weighed accurately and separately dissolved in 1 ml of dimethyl sulfoxide (DMSO) and made up the volume to 10 ml with maintenance medium. These solutions were serially diluted with maintenance medium to obtain the lower dilutions.

Procedure: The monolayer of Chang liver cells was trypsinised and the cell count was adjusted to 1.0x105 cells/ml using medium containing 10% FBS. To each well of the 96 well of micro-titer plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was removed and 100 µl of different extract concentrations was added to the cells in microtiter plates. The plates were then incubated at 37 °C for 72 h in 5% CO2 atmosphere, and microscopic examination was carried out and observations recorded every 24 h. After 72 h, the extract solutions in the wells were discarded and 50 µl of freshly prepared MTT (2 mg/ml, prepared in PBS) was added to each well [44]. The plates were gently shaken and incubated for 3 h at 37 °C in 5% CO2 atmosphere. After 3 h, the supernatant was removed and the formazan crystals formed in the cells were solubilized by addition of 50 µl of isopropanol. The absorbance was measured using a micro-plate reader at a wavelength of 540 nm. The percentage cell death was calculated using the formula given below and CTC50 values were calculated by plotting a graph for concentration against % cell death.

% cell death = control absorbance-sample absorbance / Control absorbance × 100

***In-vitro* Hepatoprotective Activity against Paracetamol Induced Toxicity:** Below the CTC50 value three dose levels were selected for each drug sample and used for further studies. The monolayer of Chang liver cells was trypsinized and the cell count was adjusted to 1.0x105 cells/ml using medium containing 10% newborn calf serum. To each well of the 96 well microtiter plate, 100µl of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once with PBS (pH 7.4) and treated with 100µl of different extract concentrations for 24h.After 24h, the cells were challenged with 100µl of 50mM paracetamol. The plates were then incubated at 37°C for further 48 h in 5% CO2 atmosphere. After 72 h, the drug solutions in the wells were discarded and 50µl of MTT in (2 mg/ml in PBS, pH 7.4) was added to each well.

The plates were gently shaken and incubated for 3 h at 37 °C in 5% CO2 atmosphere. The supernatant was removed and 50µl of isopropanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a micro-plate reader at a wavelength of 540 nm. The percentage cell viability was calculated using the formula as mentioned above.

RESULTS AND DISCUSSION:

***In-vitro* Antioxidant Activity:** In case of *Aegles marmelos* acetone and methanolic extract showed better antioxidant activity with an IC50 value of 154 ± 13µg/ml and 181 ± 0.21 µg/ml respectively (**Table 1 and Fig. 1**).

Chloroform, acetone and methanol extract of *Lowsonia innermis* showed significant DPPH radical scavenging activity with an IC50 value of 167± 1.9 µg/ml, 21 ± 0.05 µg/ml and 37 ± 0.29 µg/ml respectively (**Table 2 and Fig. 2**).

On the other hand, only the methanol extract of *Murraya koenigii* showed better DPPH radical scavenging activity with an IC50 value of 137 ± 1.9µg/ml (**Table 3 and Fig. 3**).

Acetone, methanol and petroleum ether extract of *Aegles marmelos* showed higher ABTS radical scavenging activity with an IC50 value of 43 ± 0.19, 83 ± 0.13 and 131 ± 1.7 µg/ml respectively. Acetone, methanol, aqueous and chloroform extract of *Lowsonia innermis* showed very promising ABTS radical scavenging activity with an IC50 value of 13 ± 0.12, 31 ± 1.9, 36 ± 1.2 and 133 ± 3.7 µg/ml respectively. Aqueous, methanol and acetone extracts of and *Murraya koenigii* showed the IC50 value of 121 ± 1.3, 137 ± 2.1 and 198 ± 1.7 µg/ml respectively. Trolox as a standard antioxidant compound has showed the highest ABTS scavenging activity with an IC50 of 3.1 µg/ml.

The results of O-phenanthroline assay for *Aegles marmelos* are shown in **Table 1** and **Fig. 1C**. Here also as found in DPPH and ABTS radical scavenging, the acetone (IC50 31 ± 0.17 µg/ml) and methanol (IC50 59 ± 0.07 µg/ml) extract of *Aegles marmelos* showed maximum antioxidant capacity followed by aqueous (IC50 102 ± 1.8 µg/ml) and chloroform (IC50 201 ± 2.8 µg/ml) extract.

In case of *L. innermis* the order of antioxidant activity by O-phenanthroline assay was found to be acetone > methanol > chloroform > aqueous with corresponding IC50 value of 22 ± 3.5, 52 ± 1.9, 52 ± 0.25 and 208 ± 1 µg/ml. Only the aqueous (IC50 190 ± 0.6 µg/ml) and methanol (IC50 195 ± 7.5 µg/ml) extract of *Murraya koenigii* was able to show some antioxidant capacity with O-phenanthroline assay.

Petroleum ether and acetone extract of *Aegles marmelos* showed highest total antioxidant capacity at 1000 µg/ml concentration (33 ± 0.07 mg/g and 11 ± 0.07 mg/g Trolox equivalent). In *Lowsonia innermis* the highest total antioxidant capacity was showed by chloroform (47 ± 0.31 mg/g), petroleum ether (38 ± 2.7 mg/g), acetone (31 ± 0.63 mg/g) and methanol (17 ± 0.39 mg/g). While in case of *Murraya koenigii* the order of activity was acetone (57 ± 0.51 mg/g), petroleum ether (36 ± 0.29 mg/g), chloroform (21± 0.19 mg/g) and methanol (17 ± 0.3mg/g) (**Table 4 and Fig. 4**).

Interestingly compared to other three antioxidant assays; in total antioxidant assay the petroleum ether extract of all the three plants has showed somewhat higher antioxidant capacity. This shows that the antioxidant activity of plant extracts is not limited to phenolics, and it may be due to the other antioxidant secondary metabolites, such as volatile oils, carotenoids and vitamins.

The results showed that moderate correlation exist between the total polyphenol content and their antioxidant activity with the exception of *Aegles marmelos* which showed better correlation (Pearson's r = - 0.975) in O-phenanthroline assay (**Table 5**). The lack of correlation in other plant extracts may be due the fact that specific phenolic are responsible for their antioxidant activity, other secondary metabolites may be responsible for the antioxidant activity and existence of synergisms between phenolic compounds and other secondary metabolites [45].

TABLE 1: IC50 VALUES (µG/ML) OF DIFFERENT EXTRACTS OF *AEGLES MARMELOS* IN DIFFERENT ANTIOXIDANT ASSAY MODELS

Extract	Antioxidant assay		
	DPPH	ABTS	O-phenanthroline
Petroleum ether	479 ±15	131 ±1.7	353 ±2.3
Chloroform	>1000	235 ±1.7	201 ±2.8
Acetone	154 ±13	43 ±0.19	31 ±0.17
Methanol	181 ±0.21	83 ±0.13	59 ±0.07
Aqueous	>1000	221 ±0.40	102 ±1.8
Ascorbic acid	4.1 ±0.04	-	14 ±0.23
Trolox	-	3.1 ±0.008	-

Values are expressed as Mean±Sem of three determination.

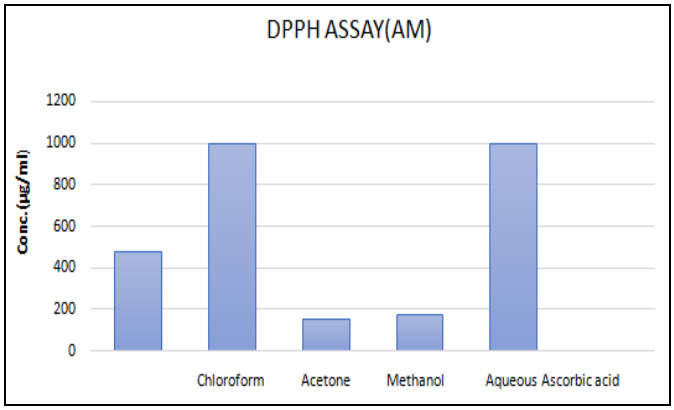

FIG. 1A: DPPH RADICAL

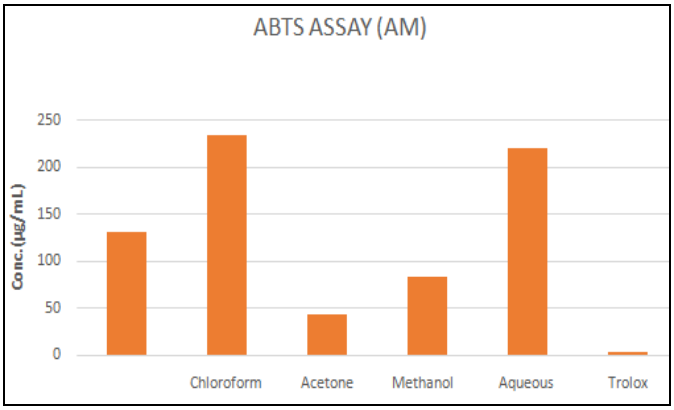

FIG. 1B: ABTS RADICAL

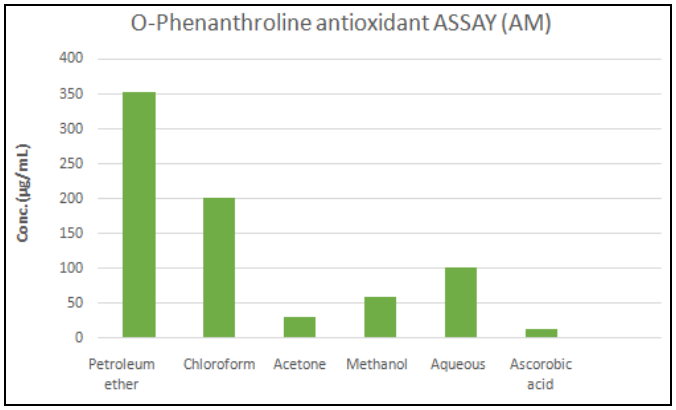

FIG. 1C: O-PHENANTHROLINE ANTIOXIDANT ASSAY

FIG. 1: IC_{50} VALUES (µG/ML) OF DIFFERENT EXTRACTS OF *AEGLES MARMELOS* (A) DPPH RADICAL; (B) ABTS RADICAL AND (C) O-PHENANTHROLINE ANTIOXIDANT ASSAY METHOD

TABLE 2: IC50 VALUES (µG/ML) OF DIFFERENT EXTRACTS OF *LOWSONIA INNERMIS* IN DIFFERENT ANTIOXIDANT ASSAY MODELS

Extract	Antioxidant assay		
	DPPH	ABTS	O-phenanthroline
Petroleum ether	818 ±38	397 ±2.2	96 ±1.7
Chloroform	174 ±1.3	133 ±3.7	52 ±0.23
Acetone	21 ±0.05	13±0.12	22 ±3.7
Methanol	37 ±0.29	31 ±1.9	52 ±1.9
Aqueous	544 ±12	36±1.2	208 ±1.0
Ascorbic acid	4.1 ±0.04	-	14 ±0.23
Trolox	-	3.1 ±0.008	-

Values are expressed as mean ± SEM of three determination.

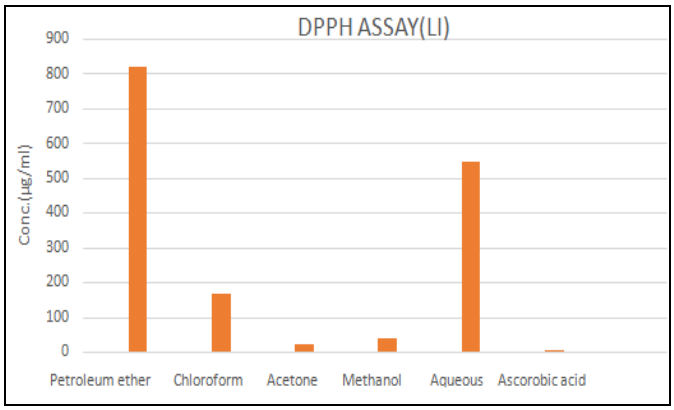

FIG. 2A: DPPH RADICAL

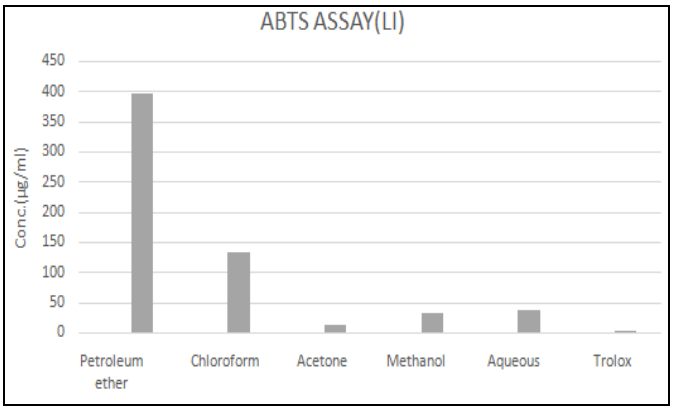

FIG. 2B: ABTS RADICAL

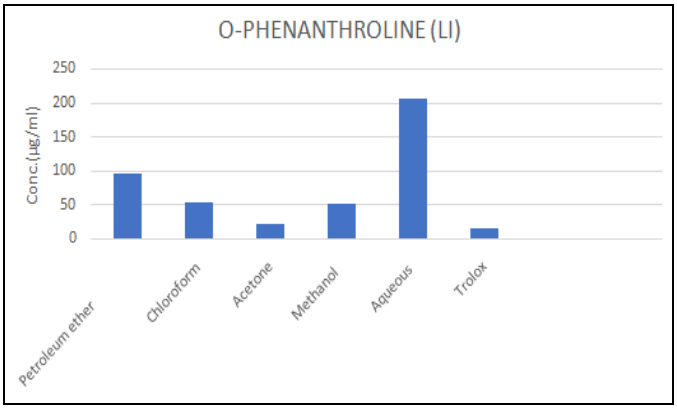

FIG. 2C: O-PHENANTHROLINE ANTIOXIDANT ASSAY METHOD

FIG. 2: IC50 VALUES (µG/ML) OF DIFFERENT EXTRACTS OF *LOWSONIA INNERM* IS (A) DPPH RADICAL; (B) ABTS RADICAL AND (C) O-PHENANTHROLINE ANTIOXIDANT ASSAY METHOD

TABLE 3: IC50 VALUES (µG/ML) OF DIFFERENT EXTRACTS OF *MURRAYA KOENIGII* IN DIFFERENT ANTIOXIDANT ASSAY MODELS

Extract	Antioxidant assay		
	DPPH	ABTS	O-phenanthroline
Petroleum ether	>1000	>1000	359 ±8.7
Chloroform	733 ±19	615 ±0.81	85 ±1.7
Acetone	468 ±0.79	198 ±1.7	379 ±2.5
Methanol	137 ±1.9	137 ±2.1	197 ±7.1
Aqueous	>1000	121 ±1.3	192 ±0.7
Ascorbic acid	4.1 ±0.04	-	14 ±0.23
Trolox	-	3.9 ±0.007	-

Values are expressed as mean ± SEM of three determination.

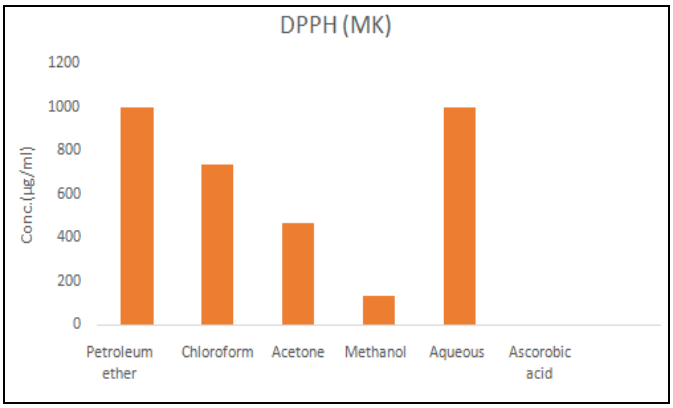

FIG. 3A: DPPH RADICAL

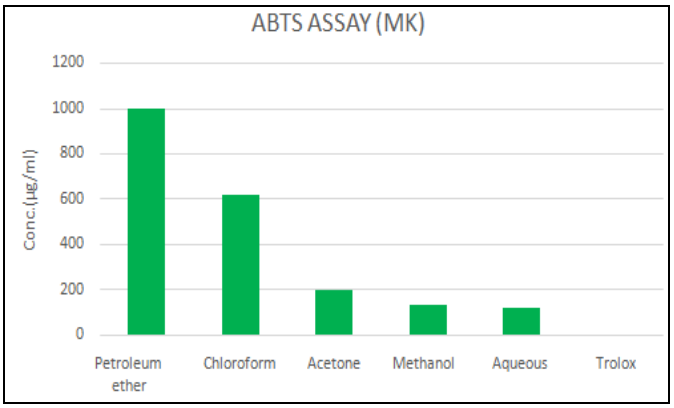

FIG. 3B: ABTS RADICAL

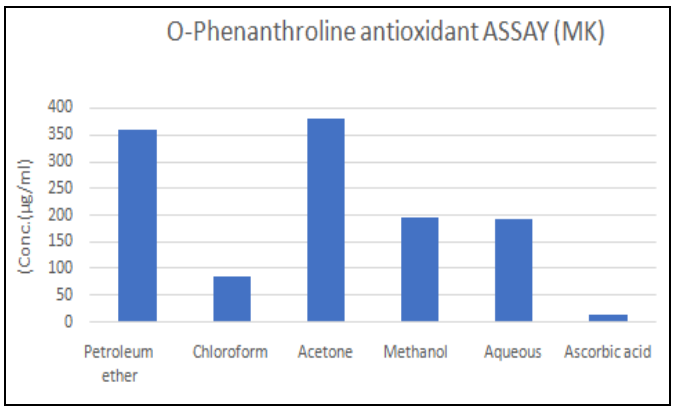

FIG. 3C: O-PHENANTHROLINE ANTIOXIDANT ASSAY METHOD

FIG. 3: IC50 VALUES (µG/ML) BY DIFFERENT EXTRACTS OF MURRAYA KOENGIIIN (A) DPPH RADICAL; (B) ABTS RADICAL AND (C) O-PHENANTHROLINE ANTIOXIDANT ASSAY METHOD

TABLE 4: TOTAL ANTIOXID ANTCAPACITY OF DIFFERENT EXTRACTS OF *A. MARMELOS, L. INNERMIS* AND *M. KOENIGII* (MG/G TROLOX EQUIVALENT

Extract	*A. marmelos*	*L. innermis*	*M. koenigii*
Petroleumether	33 ±0.07	38±2.7	36 ±0.29
Chloroform	2 ±0.12	47 ±0.31	21 ±0.19
Acetone	11±0.07	31 ±0.63	57 ±0.51
Methanol	8.4±0.12	17 ±0.39	17 ±0.3
Aqueous	2 .8 ±0.09	8.9 ±0.23	3.6 ±0.13

TABLE 5: PEARSON'S CORRELATION(R) OF TOTAL POLYPHENOLIC CONTENT (TPC) VERSUS VARIOUS ANTIOXIDANT ASSAYS OF *A. MARMELOS, L. INNERMIS* AND *M. KOENIGII*

Plant	r(TPCvs DPPH)	r (TPC vs ABTS)	R (TPC vs phenanthroline)
A. marmelos	-0.391	-0.552	-0.975
L. innermis	-0.733	-0.581	-0.606
M. koenigii	-0.524	0.031	-0.074

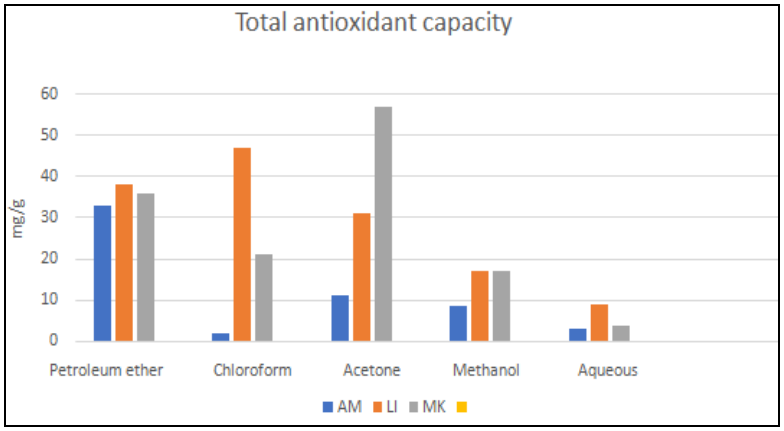

FIG. 4: TOTAL ANTIOXIDANT CAPACITY OF DIFFERENT EXTRACTS OF *A. MARMELOS, L. INNERMIS* AND *M. KOENIGII*

***In-vitro* Cytotoxicity Studies for Selected Medicinal Plant Extracts:** *In-vitro* cytotoxicity of the selected medicinal plant extracts was checked by MTT assay against normal Chang liver cells. All the tested extracts at the tested dose levels showed CTC50 value of up to or above 1000 µg/ml. This shows that the selected plant extracts are non-toxic to the liver cells (**Table 6**). Based on the cytotoxicity results, a dose of 250 µg/ml and 500 µg/ml was selected for all the extracts for further studies.

TABLE 6: *IN-VITRO* CYTOTOXICITY STUDIES OF SELECTED *A. MARMELOS* (AM), *L. INNERMIS* (LI) AND *M. KOENIGII* (MK) AGAINST CHANG LIVER CELLS

Ext/Conc.	125	250	500	1000	CTC50
AMPE	95.63±0.87	77.57±1.08	56.11±1.40	52.97±1.12	957.75
AMCHL	56.95±1.27	53.89±0.	7152.37±0.73	49.65±1.09	889.2
AMACE	96.01±1.52	60.85±2.99	51.69±0.51	49.77±1.11	828.27
AMME	97.77±0.38	78.48±2.99	54.49±1.29	52.19±0.61	906.03
AMAQ	94.93±1.71	75.08±2.41	61.36±2.77	52.37±1.08	953.99
LI PE	64.44±2.23	62.65±1.87	59.53±2.74	52.15±2.59	>1000
LI CHL	84.03±2.68	68.88±2.63	65.23±1.13	37.47±3.73	746.39
LIACE	92.49±2.97	89.58±2.21	70.94±1.57	47.63±0.24	895.23
LI ME	97.72± 0.94	94.77±1.33	89.83±1.85	54.49± 1.53	>1000
LIAQ	75.97±1.37	54.57±1.04	54.35± 0.68	50.37±1.37	>1000
MKPE	88.17±1.28	76.74±1.57	57.67±3.09	47.32±1.09	859.85
MKCHL	93.03±2.88	81.20±3.87	70.48±1.85	56.92±1.53	>1000
MKACE	92.37±2.68	63.91±1.63	54.97±2.03	49.81±1.35	858.87
MKME	77.64±1.27	68.12±2.34	63.22±2.47	58.51±1.72	>1000
MKAQ	73.69±2.75	62.58±2.43	54.29±1.63	50.28±1.43	901.57

***In-vitro* Hepatoprotective Activity of Selected Medicinal Plant Extracts against Paracetamol Intoxication:** Cells which are challenged only with paracetamol showed a percentage viability of 45%. Cells which are pretreated with extracts showed an increase in percentage viability and results are found to be significant ($p<0.05$) when compared to paracetamol challenged cells. The highest % viability was shown by acetone extract of *L. innermis* (95%) while the lowest viability was shown by petroleum ether extract of *A. marmelos* (39%, statistically not significant).All the other extracts showed dose dependent intermediate % viability thus confirming in vitro hepatoprotective potential against paracetamol challenge in Chang liver cell lines (**Table 7**).

TABLE 7: HEPATOPROTECTIVE EFFECT OF SELECTED MEDICINAL PLANT EXTRACTS ON PARACETAMOL INDUCED TOXICITY IN CHANG LIVER CELLS

Treatment	Dose		%Viability	
Control (Untreated cells)	--		100	
Paracetamol	50 mM		45 ±2.1a	
Silymarin	20µg/ml	40µg/ml	76.63±0.21b	87.43±0.43b

Extract	250µg/ml		500µg/ml	
AM Pet. ether	250 µg/ml	500 µg/ml	39.28 ± 1.79	42.73±0.37
AM Chloroform	66.84± 2.41b		81.77±0.11b	
AM Acetone	50.35± 0.73		63.87± 1.45b	
AM Methanol	43± 1.09		57.73±0.72b	
AM Aqueous	44.43± 0.37		54.59± 2.88b	
LI Pet. ether	**250 µg/ml**	**500 µg/ml**	**51.75 ± 2.38**	**50.67±0.31**
LI Chloroform	64.21±0.68b		80.27± 0.14b	
LI Acetone	92.97±2.95b		95.57±0.37b	
LI Methanol	67.99±1.05b		71.86± 1.89b	
LI Aqueous	58.94±4.69b		61.33± 3.57b	
MK Pet. ether	250 µg/ml		52.84 ± 0.79	66.47±1.05b
MK Chloroform	59.57±2.19b		62.56±1.94b	
MK Acetone	53.44±3.75		557.71±5.78 b	
MK Methanol	57.64±2.51b		58.512±1.79 b	
MK Aqueous	48.85 ± 0.78		54.28± 1.18b	

Values are mean ±S.E.M (n=3) a=p<0.05, when compared to untreated cells. b=p<0.05, when compared to paracetamol intoxicated cells.

CONCLUSION: We screened the various extracts of the selected medicinal plant *Aegles marmelos, Lowsonia innermis* and *Murraya koenigii* for their antioxidant activity by four different models. Probably due to the presence of polyphenolic compounds in them, the acetone and methanol extracts of selected medicinal plants showed better radical scavenging activity. *In-vitro* screening prior to *in-vivo* screening has now a days become a part of pharmacological evaluation of medicinal plants and it gives us some preliminary idea about the possible pharmacological effect.

In the present investigation different extracts of all the selected medicinal plants were screened for their hepatoprotective activity by using Chang liver cell lines. All the extracts of the selected medicinal plants except petroleum ether extract showed good hepatoprotective activity at the tested dose levels. This further encouraged us to screen the plants for their activity using paracetamol induced and CCl4 induced hepatotoxicity model.

both the models extract of all the selected medicinal plants showed significant hepatoprotection especially by acetone and methanol extracts that might be possibly because of higher content of phenolics and flavonoids. These polyphenolic compounds are well reputed for their diverse pharmacological activities including hepatoprotective activity. Finally, we conclude that the results of the present study demonstrate that plant *Aegles marmelos, Lowsonia innermis* and *Murraya koenigii* which were selected based on their traditional and ethnomedical claim possess potent hepatoprotective activity. Further studies are needed to reveal the possible mechanism of action.

ACKNOWLEDGMENT: We would like to thank the Head of the Department, University Department of Pharmaceutical Sciences, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India, for providing us with the opportunity and requirements needed for the accomplishment of the project.

CONFLICTS OF INTERESTS: Present study does not contain any conflict of interest.

REFERENCES:

1. Momoh JO, Aderele OR and Rasaq AK: Sub-acute and protective effect of *Cymbopogon citratus* against carbon tetrachloride-induced liver damage. African Journal of Biochemistry Research 2020; 14(4): 112-24.
2. Paniagua AC and Amariles P: Hepatotoxicity by drugs. Pharmacokinetics and Adverse Effects of Drugs-Mechanisms and Risks Factors 2017; 20.
3. Mardiati SM and Rahadian R: Application of Snakehead Fish to Increase Liver Function of Rats after Experiencing Physiological Stress. Biosaintifika: Journal of Biology & Biology Education 2018; 10(1).
4. Khalid SA, Farouk A, Geary TG and Jensen JB: Potential antimalarial candidates from African plants: An *in-vitro* approach using *Plasmodium falciparum*. J Ethnopharmacol 1986; 15: 201-209.
5. Lemma MT, Ahmed AM, Elhady MT, Ngo HT, Vu TL, Sang TK, Campos-Alberto E, Sayed A, Mizukami S, Na-Bangchang K and Huy NT: Medicinal plants for *in-vitro* antiplasmodial activities: A systematic review of literature. Parasitology International 2017; 66(6): 713-20.
6. Gandhi PR, Kamaraj C, Vimalkumar E and Roopan SM: *In-vivo* antiplasmodial potential of three herbal methanolic extracts in mice infected with Plasmodium berghei NK65. Chinese Herbal Medicines 2019; 11(3): 299-307.
7. Parekh S and Soni A: Nyctanthesarbor-tristis: Comprehensive review on its pharmacological,

antioxidant, and anticancer activities. Journal of Applied Biology and Biotechnology 2020; 8(1): 95-104.
8. Chandra K, Khan W, Jetley S, Ahmad S and Jain SK: Antidiabetic, toxicological, and metabolomic profiling of aqueous extract of *Cichorium intybus* seeds. Pharmacognosy Magazine 2018; 14(57): 377-83.
9. Elbestawy MK, El-Sherbiny GM and Moghannem SA: Antibacterial, antibiofilm and anti-inflammatory activities of eugenol clove essential oil against resistant Helicobacter pylori. Molecules 2023; 28(6): 2448.
10. Ma N, Yang Y, Liu X, Kong X, Li S, Qin Z, Jiao Z and Li J: UPLC-Q-TOF/MS-based metabonomic studies on the intervention effects of aspirin eugenol ester in atherosclerosis hamsters. Scientific Reports 2017; 7(1): 10544.
11. Madaan R, Singla RK, Kumar S, Dubey AK, Kumar D, Sharma P, Bala R, Singla S and Shen B: Bergenin-a biologically active scaffold: Nanotechnological perspectives. Current Topics in Medicinal Chemistry 2022; 22(2): 132-49.
12. Basu D and Sen R: Alkaloids and coumarins from root-bark of *Aegle marmelos*. Phytochemistry 1974; 13: 2329-30.
13. Rafe MR: A review of five traditionally used anti-diabetic plants of Bangladesh and their pharmacological activities. Asian Pacific Journal of Tropical Medicine 2017; 10(10): 933-9.
14. Choudhuri MS, Khan MT and Gambari R: *In-vitro* antiproliferative effect on human tumor cell lines of extracts from the Bangladesh medicinal plant Aegle marmelos Correa. Phytomedicine 2003; 10: 300-8.
15. Demurtas M, Baldisserotto A, Lampronti I, Moi D, Balboni G, Pacifico S, Vertuani S, Manfredini S and Onnis V: Indole derivatives as multifunctional drugs: Synthesis and evaluation of antioxidant, photoprotective and antiproliferative activity of indole hydrazones. Bioorganic Chemistry 2019; 85: 568-76.
16. Sharma N, Radha, Kumar M, Zhang B, Kumari N, Singh D, Chandran D, Sarkar T, Dhumal S, Sheri V and Dey A: *Aegle marmelos* (L.) Correa: an underutilized fruit with high nutraceutical values: a review. International Journal of Molecular Sciences 2022; 23(18): 10889.
17. Hasan MS, Ahmed MI, Mondal S, Uddin SJ, Masud MM and Sadhu SK: Antioxidant, antinociceptive activity and general toxicity study of Dendrophthoe falcate and isolation of quercetin as the major component. Orient Pharm Exp Med 2006; 6: 355- 60.
18. Mohesh G, Pradeep P, Rashmi KS, Meerasa SS and Govindasamy V: Effect of methanolic extract of *Dendrophthoe falcata* leaves on acute hyperglycemic adult zebrafish. National Journal of Physiology, Pharmacy and Pharmacology 2022; 12(10): 1692-7.
19. Alisi CS, Onyeze and GOC: Nitric oxide scavenging ability of ethyl acetate fraction of methanolic leaf extracts of *Chromola enaodorata* (Linn.). African Journal of Biochemistry Research 2008; 2(7): 145-50.
20. Hosseini Nia E, Arabi S and Hekmati M: Comparative evaluation of different extraction methods for the assay of phytochemicals and antioxidant activity of *Valeriana officinalis* roots. Journal of Medicinal Herbs 2019; 10(3): 87-93.
21. Donkor Sarkodie C, Quainoo AK and Gustav M: Propagation of Henna (*Lawsonia inermis*) cuttings using nathelene acetic acid, indole-3- butyric acid and wood ash. JPBAS 2013; 1: 115-23.
22. Mohamed MA, Eldin IM, Mohammed AE and Hassan HM: Effects of *Lawsonia inermis* L. (Henna) leaves' methanolic extract on carbon tetrachloride-induced hepatotoxicity in rats. Journal of Intercultural Ethnopharmacology 2016; 5(1): 22.
23. Varghese JK, Silvipriya K, Resmi S and Jolly C: *Lawsonia inermis* (Henna): A natural dye of various therapeutic uses - A review. Inventi Rapid Cosmeceuticals 2010; 1: 1-5.
24. Abdulmoneim Saadabi M: Evaluation of *Lawsonia inermis* Linn. (Sudanese Henna) leaf extracts as an antimicrobial agent. Res J Biol Sci 2007; 2: 419-23.
25. Chaudhary G, Goyal S and Poonia P: *Lawsonia inermis* Linnaeus: A phytopharmacological review. Int J Pharm Sci Drug Res 2010; 2: 91-8.
26. Muheyuddeen G, Rav DS, Verma S, Gautam SK and Gupta SK: *Lawsonia inermis* linnaeus: Pharmacological peculiarity and modern progression. Research Journal of Pharmacognosy and Phytochemistry 2023; 15(1): 63-76.
27. Anonymous. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. Publication & Information Directorate, New Delhi: CSIR, 1998; 446-448.
28. Batiha GE, Teibo JO, Shaheen HM, Babalola BA, Teibo TK, Al-Kuraishy HM, Al-Garbeeb AI, Alexiou A and Papadakis M: Therapeutic potential of *Lawsonia inermis* Linn: a comprehensive overview. Naunyn-Schmiedeberg's Archives of Pharmacology 2023; 1-6.
29. Prajapati ND, Purohit SS, Sharma AK and Kumar T: A Handbook of Medicinal Plants. Jodhpur: Agrobios 2003; 352-353.
30. Singh AG: *Murraya koenigii* (L.) Spreng-Curry Leaves/MithoNim-A Miracle Plant. Butwal Campus Journal 2020; 3(1): 125-30
31. Farnsworth NR: Ethnopharmacology and Drug Development. In: Ethnobotany and the search for new drugs, Wiley, Chichester (Ciba Foundation Symposium 1994; 185.
32. Sindhu RK and Arora S: Phytochemical and Pharmacognostical studies on *Murraya koenigii* L. spreng roots. Drug Invention Today 2012; 4: 325-336.
33. Kumar D and Bhan V: Impact of *Murraya koenigii* (L.) Sprengel (Rutaceae) extracts on lifespan and oxidative stress markers in *Drosophila melanogaster* Meigen, 1830 (Diptera). Brazilian Journal of Biological Sciences 2020; 7(15): 59-67.
34. Kurain JC: In: Plants That Heal, Oriental Watchman Publishing House, Pune, India 1995; 296.
35. Aju BY, Rajalakshmi R and Mini S: Evaluation of antioxidant activity of *Murraya koenigii* (L.) Spreng using different *in-vitro* methods. Journal of Pharmacognosy and Phytochemistry 2017; 6(4): 939-42.
36. Shimada K, Fujikawa K, Yahara K and Nakamura T: Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry 1992; 40(6): 945-8.
37. Akgül H, Mohammed FS, Kına E, Uysal İ, Sevindik M and Doğan M: Total Antioxidant and Oxidant Status and DPPH Free radical activity of *Euphorbia eriophora*. Turkish Journal of Agriculture-Food Science and Technology 2022; 10(2): 272-5.
38. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C: Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 1999; 26(9-10): 1231-7.
39. Byun NY, Heo MR and Yim SH: Correlation of anti-wrinkling and free radical antioxidant activities of Areca nut with phenolic and flavonoid contents. Food Science and Technology 2021; 41: 1041-9.

40. Liu P, Li Q, Wang H, Lu T, Li Y, Sui X, Yu H and Jiang W: Ethylene signaling plays an important role in UV-B-induced ascorbic acid accumulation in *Cucumis sativus* leaves. Authorea Preprints 2022; 22.
41. Capanoglu E, Kamiloglu S, DemirciCekic S, Sozgen Baskan K, Avan AN, Uzunboy S and Apak R: Antioxidant activity and capacity measurement. Plant Antioxidants and Health 2020; 1-66.
42. Pan Y, Wang K, Huang S, Wang H, Mu X, He C, Ji X, Zhang J and Huang F: Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus longan* Lour.) peel. Food Chemistry 2008; 106(3): 1264-70.
43. Prieto P, Pineda M and Aguilar M: Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry 1999; 269(2): 337-41.
44. Mosmann T: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J of Immunological Methods 1983; 65(1-2): 55-63.
45. Stagos D, Amoutzias GD, Matakos A, Spyrou A, Tsatsakis AM and Kouretas D: Chemoprevention of liver cancer by plant polyphenols. Food and Chemical Toxicology 2012; 50(6): 2155-70.

How to cite this article:
Barik S and Panda PK: *In-vitro* evaluation of anti-oxidant and hepatoprotective activity of *Aegle marmelos*, *Lawsonia innermis* and *Murraya koengii*. Int J Pharm Sci & Res 2024; 15(6): 1859-69. doi: 10.13040/IJPSR.0975-8232.15(6).1859-69.