Egypt. J. Chem. **Vol. 64,** No. 9 pp. 4747 - 4756 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Bioactive Compounds From Mango Peels (*Mangifera Indica* Tommy Atkins) And Demonstration Of Its Cytotoxicity And CCl_4 Induced Hepatotoxicity In Rats

Sahar A.M Hussein[a]*, Amani M.D.El-Mesallamy [b], Abdelmohsen M.Soliman[c], Mohamed I.M. El-Zaidy[b] , Mohamed El-Garby Younes[b]

[a]*Department of Phytochemistry and Plant Systematics; National Research Centre, Giza, Egypt.*
[b]*Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt.*
[c]*Department of Therapeutic Chemistry; National Research Centre, Giza, Egypt.*

Abstract

Mangifera indica (*Tommy Atkins*) peels, commonly known as mango, is a pharmacologically, ethnomedically, and phytochemically diverse plant. Peels is a major by-product during processing of mango fruit into pulp. In the present study, Thirteen pure bioactive compounds were isolated from methanolic peels extract. Six of them are new ellagitannins,namely, 1,2,3,4,6 -Penta-*O*-galloyl-ß-$^4C_1$-glupyranose **(3);** 2,3,6-Tri-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose**(4)**; 2,3-Di-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose,Nilocitin**(6);**3,6-Di-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose**(8)**;1,6 -Di-*O*-galloyl- β-$^4C_1$-glucopyranose **(9) ;** 1,3-Di-*O*-galloyl-β-$^4C_1$-glucose **(11)**, which were analyzed for the first time from *M. indica* (*Tommy Atkins*) peels. The ameliorative effect of the methanolic extract of *M. indica* (*Tommy Atkins*) peels towards the CCl_4-induced hepatotoxicity in male Wistar rats through measuring certain biochemical parameters content in the liver were analyzed. The CCl_4-treated rats showed a significant decline in the studied the serum levels of high-density lipoprotein (HDL), albumin (A) as well as the hepatic levels of glutathione (GSH) and activities of catalase (CAT), superoxide dismutase (SOD) , glutathione reductase (GR), elevation in the levels of total lipids (TL), triglycerides (TG), total cholesterol (TC), low-density lipoproteins (LDL), globulin (G), total bilirubin (TBil) , alanine and aspartate aminotransferase and alkaline phosphatase (ALAT and ASAT, ALP) and the hepatic levels of malondialdehyde (MDA). In contrast, the administration of methanol extract, notably improved all the studied parameters. This study showed that CCl_4 administration to Wistar rats, at a high dose level, could induce a hepatic injury in addition to certain metabolic alterations. The work was extended to investigate tissue histopathology.

Thus, results suggest that the peels extract can be a potential source of an attractive candidate for ameliorating of hepatotoxicity induced by CCl_4 through scavenging free radicals, improved liver functions, and normalizing the liver histopathological architecture.

Keywors: Mangifera indica (Tommy Atkins) peels, Phenolics, gallotannines, NMR spectroscopy, In *Vivo* Hepatoprotective activity

Introduction:

M. indica (*Tommy Atkins*). is commonly referred to as mango. The genus *Mangifera* belongs to the family Anacardiaceae. Genus *Mangifera* approximately contains 69 different species with *M. indica* being the most common species in the same genus. *M. indica* plant is an evergreen broad canopy tree which grows to a height of 8–40 meter. In Egypt, mangoes grow in coastal areas of Delta Nile. They grow best in tropical regions. The soil and climate of Ismailia and Sharqia, are especially favorable to the cultivation of Egyptian mango. A large variety of chemical compounds have been reported in *M. indica* [1]. Phytochemicals in *M. indica* are polyphenols (flavonoids and phenolic acids) are the most abundant compound types in *M. indica* [2]. Mangiferin, gallic acid, ellagic acids, catechins, quercetin, kaempferol, protocatechuic acid, methyl gallate and anthocyanins are the major polyphenolic compounds found in *M. indica* [3]. The quantities of polyphenols in mango depend on the part and variety of mango [4]. The amount of polyphenols is high in many parts of *M. indica*. Thus, a pure compound alone has been proven to be less effective than crude drugs, implying that the synergism of many *M. indica*. Polyphenols is essential for optimum biological activities [5,6]. Extracts of *M. indica* (*Tommy Atkins*) peels have been reported to possess antiviral, antibacterial, analgesic, anti-inflammatory, and

*Corresponding author e-mail: drsahar90@yahoo.com.; (Sahar A.M Hussein).
Receive Date: 22 April 2021, Revise Date: 27 April 2021, Accept Date: 04 May 2021
DOI: 10.21608/EJCHEM.2021.73347.3632

immune-modulatory activities [7]; *in vitro* anti amoebic activity [8]. In traditional medicine, the use of mango extracts as herbal drugs is widespread. There are several reports available concerning the traditional uses of mango in various parts of the world. Some phenolic compounds present in mango are antioxidants, contributing to a reduction in the risk of cardiovascular diseases, while others such as gallic acid [9] and quercetin [10,11] are claimed to have activity against allergies, inflammation, hypertension, arthritis, and carcinogenesis [12,13].

For that reason, further information on phenolic structures characterization in mango culmination would make a contribution to a higher knowledge in their implications in the fruit's quality as a source of dietary compounds with potential biological properties.

Unlike the immuneostimulant, anticancer and antimicrobial marketers synthesized with the aid of using chemical sources, those from herbal sources are readily ordinary with the aid of using the consumers.

In the present investigation, we present the isolation of bioactive phenolic compounds from *M. indica* (*Tommy Atkins*) peels as well as their hepatoprotective effect towards carbon tetrachloride CCL_4-induced injury in rat's liver. These mango cultivars, which are normally consumed in Egypt, can turn out to be a brand-new supply for the pharmaceutical industry.

Materials and Methods:

Preparation of Peels Powders:

Fresh mangoes were collected from a particular mango tree (2 kg) between June and August, 2019, from Abu Hammad- Zagazig - Sharqia, Egypt which were identified and authenticated as *M. indica (Tommy Atkins)* peels by a taxonomist in the department of Botany, Faculty of Science, Zagazig University, Egypt.

The *M. indica* (*Tommy Atkins*) were washed with warm water and peels were recovered by using sharp knife and the peels were washed with distilled water and dried. The dried peels were ground using mixer and grinder to the formation of a course powder. The *M. indica* (*Tommy Atkins*) peels powder was stored at 4ºC for further analysis.

Extraction of *M. indica* (*Tommy Atkins*) Peels.:

Fresh dried *M. indica* peels (250 gm) were extracted with (500 ml) petroleum ether 60-80º, followed by (500 ml) *n*-hexane to remove lipids, then (500 ml) ethyl acetate, finally by methanol (500 ml) by using Soxhlet apparatus. The extraction processes carry on till the solvent in siphon tube of Soxhlet apparatus become colorless, the extracts were then filtered using filter papers and concentrated under vacuum on a rotary evaporator. Yielded extracts were 6.78, 4.40 and 2.32 gm, respectively. The dried plant crude extracts kept in refrigerator for further analysis.

Chemicals Reagent:

All standards (Ellagic acid, gallic acid, chlorogenic acid, mangiferin, protocatechuic acid, *p*-coumaric acid, vanillic acid, ferulic acid, methyl gallate, (+)-catechin, rutin and kaempferol) were used for identification and quantification were purchased from Sigma-Aldrich, and solvents used purchased from Merck (Germany)

General

NMR spectra were acquired in DMSO-d_6 or (CD_3OD), on a Jeol ECA 400 MHz NMR spectrometer,^{1} H at 400 MHz and ^{13}C 100 MHz, (Zagazig University). Standard pulse sequence and parameters were used to obtain one-dimensional. ^{1}H chemical shifts (δ) were measured in ppm, relative to TMS ,^{13}C - NMR chemical shifts to DMSO-d_6 by adding 39.5 ppm . High resolution ESI mass spectra were measured using a Finnigan LTQFT Ultra mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). UV spectra were recorded on a Shimadzu UVVisible-1601 spectrophotometer (Kyoto, Japan). Paper chromatographic analysis was carried out on Whatman 1 MM and 3 MM, using solvent systems: (1) H_2O; (2) 6% Ac OH; (3) BAW (*n*-Bu OH–AcOH–H_2O, 4:1:5, upper layer).

Isolation and Identification of Bioactive Phenolics

The obtained methanol peels extract was concentrated under vacuum at 40°C. (2.32 gm) was applied to a polyamide S_6 column, which was eluted with distilled water (500 ml) then with H_2O/MeOH by decreasing polarities to yield thirteen sub fractions (1-13), removal of the solvents were individually collected and purified. Compounds 1,2,3 (55 ,45,91 mg) were isolated pure from fraction II. Compounds 4,5,6 (65,34,47mg) were individually separated pure by fractionation of fraction III over Sephadex LH-20 column using a H_2O/MeOH mixture with decreasing polarity for elution. Compounds 7,8,9(48,56,38 mg) were individually isolated pure from fraction IV by fractionation on a Sephadex LH-20 column and 40% H_2O/MeOH for elution, followed by preparative paper chromatography (prep. PC), using BAW system for final purification. Compounds 10,11 (45,56 mg) were individually separated pure from fraction V by (prep. PC), using *n*-BuOH water saturated as solvent. Compound 12,13 (75, 58 mg) were individually isolated pure from fraction VI by fractionation on a polyamide column using (methanol: benzene: H_2O) (60: 38: 2), followed by (prep. PC). The chemical structures have been established by conventional methods of chemical and physical analysis and confirmed by ^{1}H and ^{13}C-NMR spectroscopy.

Assessment of Biological Activities

In *Vivo* Hepatoprotective Activities

Animals

Male Wistar albino rats (120 to 150 g) were selected for this study. They were obtained from the Animal House, National Research Center, Egypt. All animals were kept in controlled environment of air and temperature with access of water and diet ad libitum. Anesthetic procedures and handling with animals complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt.

Experimental Design

Male rats (18) were used in this study. Animals were divided into 3 groups: **Group 1**, served as normal healthy control rats. **Group 2**, Rats were intraperitoneally injected with 500 microliters of CCl4 diluted 1: 9 (v/v) in olive oil (0.1 ml) twice a week for six consecutive weeks. **Group 3**,

Rats were intraperitoneally injected with CCl_4 (0.1 ml) followed by oral administration of *M. indica* methanolic extract (200 mg/kg body weight).

Study of Some Biochemical Parameters
Sample Preparations

Blood was collected from each animal by puncture of sublingual vein. Blood samples were collected into dry test tubes and then centrifuged at 3000 rpm in order to separate serum. The sera were kept at -20°C for further biochemical analysis. In order to collect the hepatic tissues, rats were immediately dissected. The liver was homogenized with 10% w/v ratio in ice-cold 50 mM Tris HCl buffer at pH 7.4 and then centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was collected and kept in deepfreeze at -20°C for further analyses.

Estimation of Serum Biochemical Parameters

In the serum of all the experimental groups, the levels of total lipids (TL), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total proteins (TP), albumin (A), globulin (G), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), total bilirubin (TBil) and direct bilirubin (DBil) were measured colorimetrically using Biodiagnostics kits (Dokki, Giza, Egypt).

Non-enzymatic and Enzymatic Antioxidant Assay

Lipid peroxide assay: The level of malondialdehyde (MDA) in the liver homogenate was assayed according to the described technique [14]. The principle of this method depends on the reaction of the liberated MDA after lipid peroxidation (LPO) of the cell membranes with thiobarbituric acid in acidic medium.

The concentrations of non-enzymatic (glutathione, GSH) as well as enzymatic (catalase, CAT, superoxide dismutase, SOD, glutathione reductase, GR) antioxidants were estimated in the homogenate of the liver of control and treated rats. The method by which GSH content was measured was based on the reaction of 5, 5'-Dithiobis-2-nitrobenzoic acid with GSH [15]. The CAT activity was estimated in accordance to the method described [16]. The SOD activity assessment was based on the ability of SOD to inhibit the reduction reaction of nitro blue tetrazolium dye mediated by phenazine methosulphate [17]. The principle for measuring the GR activity was based on its ability to catalyze the reduction of glutathione (GSSG) [18].

Histopathological Study

Liver tissues were excised from sacrificed animals, individually weighed, and, from them, 5 µm thickness slices were cut, fixed in 10% paraformaldehyde, and embedded in paraffin wax blocks. Tissue sections of 5 µm thick were stained with hematoxylin and eosin (H&E).

Statistical Analysis

Data were statistically analyzed by the aid of Statistical Package of the Social Sciences, SPSS version 23 (copyrighted by IBM SPSS software, USA). Data were expressed as a mean ± standard error of mean (SEM).

Results and Discussion

In this study, the isolation and identification of the major polyphenolic metabolites, particularly phenolic Compounds and gallotannines of methanolic extract from *M. indica* (*Tommy Atkins*) peels were carried out for the first time. Successive column chromatographic separations resulted in isolation of seven known polyphenolic acids **(1,2,5,7,10,12,13)** which were identified as; gallic acid [19]; methyl gallate [20]; ellagic acid [21]; caffeic acid [22]; chlorogenic acid [23], naringenin [24]; catechin [25], respectively, on the basis of their chromatographic properties (R_f-values, fluorescence) under UV-light and their responses towards specific spray reagents for tannins ($FeCl_3$,KIO_3). The structures of these seven metabolites were confirmed by spectroscopic analysis, including 1D-NMR, and by negative ESIMS mass as well.

Isolated Pure Phenolic Compounds And Spectroscopic Data:

1,2,3,4,6-Penta-*O*-galloyl-ß-$^4C_1$-glupyranose (3):
off-white on-crystalline powder (91 mg), negative ESI-MS analysis, m/z 939.1146 $[M-H]^-$, (calc. for $C_{41}H_{31}O_{26}$, 939.1109). ^{1}H-NMR (CD_3OD), Galloyl moieties: δ ppm 7.10, 7.04, 6.97, 6.94, 6.89 (s, each 2H), glucose moiety, δ ppm 6.23 (d, 8.3 Hz, H-1), 5.89(t, 9.6 Hz, H-3), 5.61 (t,9.6 Hz, H-4), 5.58 (dd, 9.6, 8.3 Hz, H-2), 4.40 (m, H-5),4.51 (d, 12.2 Hz, H-6α),4.37 (dd, 12.2, 4.2 Hz, H-6β). ^{13}C-NMR (CD_3OD), Galloyl moieties: 167.9, 167.3, 167.0,166.9, 166.2 (carbonyl group signals), 146.6, 146.5,146.4, 146.4, 146.3 (C-3, C-5), 140.9, 140.5, 140.4,140.2, 140.1 (C-4), 121.0, 120.3, 120.2, 120.1, 119.6(C-1), 110.6, 110.44, 110.38,110.36,110.3 (C-2, C-6). glucose moiety: δ ppm 93.9 (C-1), 74.3 (C-5), 74.1 (C-3), 72.1 (C-2), 69.8 (C-4), 63.1 (C-6).

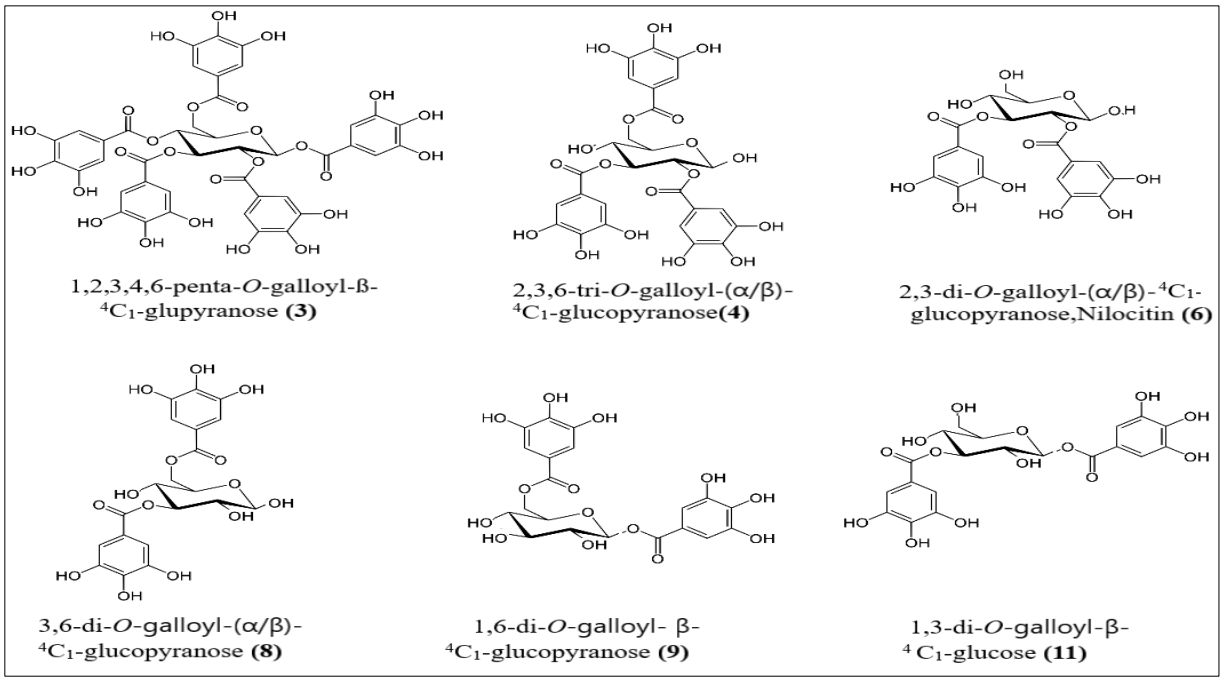
2,3,6-Tri-*O*-galloyl- (α/β)- $^4C_1$-glucopyranose (4):
Off-white amorphous powder (65mg). UV spectral data at 275 nm which is gallotannins. It exhibited a molecular ion at $[M–H]^-$: m/z= 635; HRESI-MS, negative mode: m/z = 635.4616,corresponding to a molecular formula of $C_{27}H_{24}O_{18}$ ^{1}H- NMR Spectral Data (DMSO-d_6) δ ppm α-glucose moiety : 5.37 (d, J=3.5 Hz, H-l), 4.95 (dd, J=8 and 3.5 Hz, H-2), 5.70 (t, J=8 Hz, H-3), 3.70 (m, H-4), 3.95 (m, H-5), 4.38 (d, J=12.5 Hz, H- 6), 4.25 (dd, J=12.5 Hz and 4.5 Hz, H-6').β-glucose moiety: 5.05 (d, J=8 Hz. Hl), 5.10 (t, J=8 Hz, H-2), 5.33 (t, J=8 Hz, H-3), 3.80 (m, H-4), 3.92 (m, H-5), 4.42 (d, J=12.5 Hz, H-6), 4.29 (dd,J=12.5 and 4.5 Hz, H-6').Galloyl moieties in α- and β- anomers: 7.0 (s), 6.99 (s), 6.98 (s), 6.95 (s), 6.93 (s),6.88(s).^{13}C-NMR Spectral Data (DMSO-d6) δ ppm, α-glucose moiety 91.2 (C-l),72.2 (C-2), 72.8(C-3), 69.3 (C-4), 72.3 (C-5). 64.3 (C-6). β-glucose moiety 95.3 (C-l), 73.5(C-2), 75.5 (C-3), 70.8 (C-4),74. 9(C-5), 64.4 (C-6). Galloyl moieties in α/β-anomers:121.6,121.7,121.8(C-1',C-1'',C-1'''),109.8,109.9 ,110.2 (C-2',6',C-2'',6'',C-2''',6'''), 146.1,146.0, 145.9, 14 5.4 (C-3',5',C-3'',5'',C3''', 5'''),139.3,138.6,138.8 (C-4',C-4'', C-4'''),167.0,166.8, 166.6,165.0 (C-7',C-7'',C-7''').

2,3-Di-*O*-galloyl- (α/β)-$^4C_1$-glucopyranose, Nilocitin (6):
Off-white amorphous powder (47mg) showed a blue color with $FeCl_3$, and a pink color with KIO_3 on PC indicative of gallotannins. R_f-values: 60 (H_2O), 65 (AcOH-6),50 (BAW), UV spectral data λ_{max}: 274 nm. it possess negative ESIMS spectrum $[M-H]^-$ ion peak at 483 mU corresponding to *M r* = 484, identical with those reported for di galloyl glucoses [34]. Complete acid hydrolysis yielded gallic acid and glucose (Co PC, UV spectral data,^1H,^{13}C-NMR analysis); On controlled acid hydrolysis 4 yielded only, one intermediate 4a which was separated by Prep. PC, using BAW as solvent which proved its identity as a mono galloyl glucose. ^1H- NMR Spectral Data (DMSO-d_6) δ ppm of α-glucose moiety:5.45 (1H, d, J=3.3 Hz, H-1),4.92 (1H, dd, J=8 & 3.3 Hz, H-2) ,5.78 (1H, t, J=8 Hz, H-3),3.1-4.0 (m, H-4,5,6); β-glucose moiety:4.98(1H, d, J=7.5 Hz, H-1),5.10 (1H, t, J=7.5Hz,H-2),5.41(1H, t, J=7.5Hz, H-3), 3.1-4.0 (m,H-4,5,6).Galloyl moieties in α- and β-anomers 6.87(2H,s), 6.81(4H,s), 6.79(2H, s).^{13}C-NMR Spectral Data (DMSO-d6)δ (ppm): α-glucose moiety 89.3 (C-1),73 (C-2), 73(C-3), 68.8(C-4), 73 (C-5), 60.6 (C-6) , β-glucose moiety 94.5 (C-1), 73.8(C-2), 75.5 (C-3), 68.8(C-4),76.9(C-5), 60.6 (C-6). Galloyl moieties in α-and β-anomers; 120.64, 121.38, 121.42 (C-1'α/ β',1''α/β), 109.97(C-2',6'α/β, 2'', 6''α/β),145.64 (C-3',5'α/β,3'',5''α/β),138.67, 138.9 (C-4' α/β, 4'' α/β), 164.8,165.2,165.4,165.5 (C=Oα/β, C=O α/β).

3,6-Di-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose (8):
Off-white amorphous powder (56mg). UV spectral data at 275 nm which is gallotannins. It exhibited a molecular ion at $[M–H]^-$: m/z= 483; HRESI-MS, negative mode: m/z = 483.079, corresponding to a molecular formula of $C_{20}H_{20}O_{14}$. (calc. for $C_{20}H_{20}O_{14}$,483.3572). ^1H- NMR Spectral Data (DMSO-d_6) δ ppm α-glucose moiety: 5.17 (d, J=3.5 Hz, H-l), 3.57-3.90 (m, H-2 and H-4), 5.34 (t, J=9 Hz, H-3), 3.88 (m, H-5), 4.38 (d, J=12.5 Hz, H-6), 4.25 (dd, J=12.5 Hz and 4.5 Hz, H-6'). β-glucose moiety: 4.65 (d, J=9 Hz. H-l), 3.57-3.90 (m, H-2 and H-4), 5.09 (t, J=9 Hz, H-3), 3.90 (m, H-5), 4.43 (d, J=12.5 Hz, H-6), 4.29 (dd . J=12.5 and 4.5 Hz, H-6').Galloyl moieties in α- and β-anomers: 6.94 (s), 6.96 (s), 6.99 (s), 7.01(s).^{13}C-NMR Spectral Data (DMSO-d6) δ ppm, α-glucose moiety 92.48 (C-l), 73.10(C-2), 76.30(C-3), 68.43 (C-4), 70.70 (C-5). 64.13 (C-6). β-glucose moiety 96.58 (C-l), 74.50 (C-2), 78.04 (C-3), 68.47 (C-4),74.80 (C-5), 64.40 (C-6). Galloyl moieties in α- and β-anomers: 120.21,120.59 (C-1', C-1'') , 109.22, 109.40 (C-2',6', C-2'',6''), 145.06,145.17 (C-3', 5', C-3'',5''),138.37,138.46 (C-4', C-4''), 166.99, 167.34 (C-7', C-7'').

1,6-Di-*O*-galloyl- β-$^4C_1$-glucopyranose (9)
Non-crystalline creamy white amorphous powder (38mg). Showed a blue color with $FeCl_3$, and a pink color with KIO_3 on PC indicative of gallotannins, Rf-values: 42 (H_2O),48 (HOA_C-6), 40 (BAW); UV spectral data one band at λ_{max} in MeOH: 276nm. Negative ESIMS spectrum showing peak at $[M-H]^-$ ion at m/z = 483 mU corresponding to *M r* = 484, to be a di galloyl glucose ion. On complete acid hydrolysis, yielded glucose and gallic acid (Co PC and ^1H, ^{13}C-NMR). ^1H- NMR Spectral Data (DMSO-d_6) δ ppm , allowed the definition of β- glucose protons as follows:5.52(1H, d, J=8Hz, anomeric H-1),3.2-3.7 (sugar protons overlapped with water protons, H-2, H-5) ,4.35(1H, d, J=12Hz, H-6a) ,4.2(1H, dd, J=12 and J=4.5, H-6b). Galloyl moieties in β-anomer: 6.89(2H, s), 6.95 (2H, s).^{13}C-NMR spectral analysis, afforded a spectrum containing double signals for most of the glucose and galloyl carbons. Resonances were assigned by comparison with the ^{13}C NMR data, reported for similar galloyl glucoses. β-Glucose moiety :94.9(C-1), 75.2 (C-2), 76.7(C-3), 69.8(C-4), 73.0(C-5),63.7(C-6), Galloyl moieties in β-anomer : 118.9, 119.7 (C-1',1''), 109.1,109.5(C2',6'and C2'',6''),146.0,146.1 (C3', 5' and C3'', 5''), 139, 139.5 (C-4', 4''),165.1, 166.2 (C-7', C-7'').

1,3-Di-*O*-galloyl-β-$^4C_1$- glucose (11):
Off-white amorphous powder (56mg) showed a blue color with $FeCl_3$, and a rosy red color with KIO_3 on PC indicative of gallotannins. R_f-values: 65 (H_2O),73 (Ac OH), 38 (BAW); UV spectral data one band at λ_{max} in MeOH: 278nm. Negative ESIMS spectrum showing peak at $[M-H]^-$ ion at m/z = 483mU corresponding to *M r* = 484, to be a di-galloyl glucose ion. On complete acid hydrolysis, yielded glucose and gallic acid (Co PC and ^1H, ^{13}C-NMR). ^1H-NMR Spectral Data (DMSO-d_6) δ ppm , the glucose protons as follows: 5.6 (1H, d, J=8Hz, anomeric H-1).5.03 (1H, t, J=8 Hz, H-3),3.1-4.0 (sugar protons overlapped with water protons, H-2,4,5,6). Galloyl moiety: 6.97(2H, s, H-2' and H-6'), 6.99 (2H, s, H-2'' and H-6'').^{13}C-NMR spectral analysis, afforded a spectrum containing double signals for most of the glucose and galloyl carbons. Resonances were assigned by comparison with the ^{13}C NMR data, reported for similar galloyl glucoses. Glucose moiety : 94.1(C-1), 77.1(C-2), 78.2(C-3), 70.5(C-4), 71.6(C-5),61.1(C-6), Galloyl moieties: 120.3, 119 (C-1',1''),110.1 , 110, 109, 108 (C2',6' and C2'',6'') , 145.8,145.7 (C3', 5'and C3'',5''), 138.7,139.6(C-4',4''),165.4, 166.2 (C = O).
All pure compounds were isolated from *M. indica* (Tommy Atkins) peels are carried out for the first time.



Hepatoprotective Activities
Effect on Serum Biochemical Parameters

The lipid profile of the exploratory creatures as influenced by the administration of CCl_4 alone, *M. indica* (*Tommy Atkins*) peels methanolic extract in addition to CCL_4 are appeared in **(Table 1)**. The serum levels of TL, TC, TG, LDL-C and HDL-C of the rats were especially affected by the sort of treatment. In contrast with control group, all the contemplated lipid profile parameters of CCL_4-treated group were altogether raised aside from the degrees of HDL-C that were quite diminished. Then again, rats treated *M. indica* (*Tommy Atkins*) peels methanolic extract in addition to CCL_4 showed a checked decrease in the degrees of TL, TC, TG and LDL-C, as contrasted and the CCL_4-treated group.
After the effects of the current investigation have additionally settled that CCl_4 treatment might have influenced the lipid digestion of liver (fatty oil and cholesterol levels). This is confirmed from the current perceptions in which CCl_4 caused a critical ($p < 0.05$) increment in the degrees of lipid parameters. In this association [26],CCl_4 induction is like hepatitis if there should be an occurrence of the fatty substance's catabolism. This circumstance could be additionally credited to the decrease of lipase action, which could prompt decline in fatty substance hydrolysis [27].

Then again, it very well may be expected that hypercholesterolemia in CCl_4 inebriated rats was come about because of harm of hepatic parenchymal cells that lead to aggravation of lipid digestion in liver [28]. Be that as it may, rats treated with *M. indica* (*Tommy Atkins*) peels methanolic extract demonstrated a huge ($p < 0.05$) decrease in triacylglycerol and cholesterol esteems contrasted with CCl_4-inebriated rats. The system of lipid bringing down impacts of *M. indica* (*Tommy Atkins*) peels methanolic extract may be credited to an inhibitory action on microsomal acyl coenzyme A: cholesterol acyltransferase in vitro. This chemical is answerable for acylation of cholesterol-to-cholesterol esters in liver [29].
Serum protein profile of various groups of rats in **(Table 1)** was observably influenced by the kind of treatment as rats controlled CCL_4 alone showed stamped decreases in the degrees of albumin concurrent with a critical expansion in the degrees of globulin, when contrasted with the controls. Along these lines, the A/G proportion of this group was astoundingly diminished. Then again, the rats of *M. indica* (*Tommy Atkins*) peels extract in addition to CCL_4 - treated groups showed a checked expansion in the degrees of albumin and A/G proportion however a stamped decline in the degrees of globulin, when contrasted with the CCL_4-treated group.

In this examination the critical ($p < 0.05$) decline in serum albumin of rats treated with CCl_4 when contrasted with control may shows helpless liver capacities or debilitated union, either essential as in liver cells harm or optional to decreased protein consumption and diminished assimilation of amino acids brought about by a malabsorption conditions or ailing health, or misfortune protein in pee, because of nephritic disorder and constant glomerulonephritis [30]. Then again, a huge ($p < 0.05$) increment in grouping of serum egg whites was seen in rats got *M. indica* methanolic extract in addition to CCl_4 in contrast with rats got CCl_4 alone. The expansion of albumin fixation after treatment with *M. indica* (*Tommy Atkins*) peels methanolic extract might be credited to the diminishing in lipid peroxidation cycles and expansion in the activities of plasma protein thiols because of the treatment [31].

Liver capacity markers, as impacted by the administration of CCL_4 - *M. indica* (*Tommy Atkins*) peels extract alone and blended, were introduced in **(Table 1)**. The activities of

ASAT, ALAT and ALP and TBil, in serum of rats were altogether influenced by the kind of treatment, though the serum levels of DBil were not influenced by any of the contemplated factors. In contrast with the controls, the CCL_4-treated rats demonstrated critical rises in the activities of ASAT and ALAT and ALP just as the degrees of TBil. Despite what might be expected, the activities of ALP, ASAT and ALAT just as the degrees of TBil and DBil of *M. indica* (*Tommy Atkins*) peels extract in addition to CCL_4 - treated rats were not essentially not quite the same as those of the benchmark group.

In the current investigation serum hepatic biomarkers, AST and ALT activities were extraordinarily expanded ($p < 0.05$) in rats treated with the CCl_4 contrast with control. As in the current examination, past investigations have demonstrated that $CC1_4$ expanded essentially serum ALP levels, and absolute protein and egg whites' levels [32-33]. The expanded serum levels of hepatic markers have been credited to the liver injury, in light of the fact that these proteins are found in cytoplasmic region of the cell and they are delivered into flow if there should be an occurrence of cell harm [34]. Then again, treatment with *M. indica* (*Tommy Atkins*) peels extract in addition to CCL_4 was found to stifle ($p < 0.05$) the expansion of serum AST and ALT activities. As per the current outcomes, numerous other plant removes were accounted for to have impressive restorative impacts on liver injury actuated by substance specialists, for instance, administration of poly phenolic extricates from chicory (*Cichorium intybus*) came about in completely standardization of the serum AST and ALT levels in mice presented to thioacetamide, a hepatotoxic organosulfur compound [35]. Comparative impacts from barberry extract upon administration to CCl_4 actuated hepatotoxic creatures have additionally revealed [36]. These finding infers that challenge to shield liver tissue from CCl_4 injury.

Serum protein profile of various groups of rats in **(Table 1)** was observably influenced by the kind of treatment as rats controlled CCL_4 alone showed stamped decreases in the degrees of albumin concurrent with a critical expansion in the degrees of globulin, when contrasted with the controls. Along these lines, the A/G proportion of this group was astoundingly diminished. Then again, the rats of *M. indica* (*Tommy Atkins*) peels extract in addition to CCL_4 - treated groups showed a checked expansion in the degrees of albumin and A/G proportion however a stamped decline in the degrees of globulin, when contrasted with the CCL_4-treated group.

In this examination the critical ($p < 0.05$) decline in serum albumin of rats treated with CCl_4 when contrasted with control may shows helpless liver capacities or debilitated union, either essential as in liver cells harm or optional to decreased protein consumption and diminished assimilation of amino acids brought about by a malabsorption conditions or ailing health, or misfortune protein in pee, because of nephritic disorder and constant glomerulonephritis [30]. Then again, a huge ($p < 0.05$) increment in grouping of serum egg whites was seen in rats got *M. indica* methanolic extract in addition to CCl_4 in contrast with rats got CCl_4 alone. The expansion of albumin fixation after treatment with *M. indica* (*Tommy Atkins*) peels methanolic extract might be credited to the diminishing in lipid peroxidation cycles and expansion in the activities of plasma protein thiols because of the treatment [31].

Liver capacity markers, as impacted by the administration of CCL_4 - *M. indica* (*Tommy Atkins*) peels extract alone and blended, were introduced in **(Table 1)**. The activities of ASAT, ALAT and ALP and TBil, in serum of rats were altogether influenced by the kind of treatment, though the serum levels of DBil were not influenced by any of the contemplated factors. In contrast with the controls, the CCL_4-treated rats demonstrated critical rises in the activities of ASAT and ALAT and ALP just as the degrees of TBil. Despite what might be expected, the activities of ALP, ASAT and ALAT just as the degrees of TBil and DBil of *M. indica* (*Tommy Atkins*) peels extract in addition to CCL_4 - treated rats were not essentially not quite the same as those of the benchmark group.

In the current investigation serum hepatic biomarkers, AST and ALT activities were extraordinarily expanded ($p < 0.05$) in rats treated with the CCl_4 contrast with control. As in the current examination, past investigations have demonstrated that $CC1_4$ expanded essentially serum ALP levels, and absolute protein and egg whites' levels [32-33]. The expanded serum levels of hepatic markers have been credited to the liver injury, in light of the fact that these proteins are found in cytoplasmic region of the cell and they are delivered into flow if there should be an occurrence of cell harm [34]. Then again, treatment with *M. indica* (*Tommy Atkins*) peels extract in addition to CCL_4 was found to stifle ($p < 0.05$) the expansion of serum AST and ALT activities. As per the current outcomes, numerous other plant removes were accounted for to have impressive restorative impacts on liver injury actuated by substance specialists, for instance, administration of poly phenolic extricates from chicory (*Cichorium intybus*) came about in completely standardization of the serum AST and ALT levels in mice presented to thioacetamide, a hepatotoxic organosulfur compound [35]. Comparative impacts from barberry extract upon administration to CCl_4 actuated hepatotoxic creatures have additionally revealed [36]. These finding infers that challenge to shield liver tissue from CCl_4 injury.

Non-enzymatic and Enzymatic Antioxidant Assay

Impact on the hepatic lipid peroxidation and endogenous cancer prevention agents: The impacts of CCL_4 alone or with *M. indica* (*Tommy Atkins*) peels methanolic extract administration on the degrees of hepatic MDA and GSH and the activities of endogenous cell reinforcement proteins were appeared in **(Table 2)**. The hepatic degrees of MDA and GSH just as the activities of CAT, SOD and GR were essentially impacted by the kind of treatment. In the liver of rats regulated CCL_4 alone, there was an important height in the degrees of MDA joined by a stamped decrease in the GSH substance, SOD and GR activities when contrasted with those of controls. In the rats of *M. indica* methanolic extract plus CCL_4 - treated groups, the mean estimations of hepatic MDA fixation were fundamentally lower than those of CCL_4-treated rats and were not essentially not the same as those of the controls. Then again, the mean estimations of hepatic GSH substance of *M. indica* extract in addition

to CCL_4 - treated rats were essentially higher than those of CCL_4-treated group. When contrasted with the CCL_4-treated group, the rats directed *M. indica* extract in addition to CCL_4 indicated a stamped height in the activities of CAT and SOD and GR, that didn't essentially contrast from those of the controls.

Information of the current investigation is as per the discoveries of different workers, for example, the research of scientist [37] who revealed that hepatotoxic impacts by CCl_4 are lipid peroxidation root and are to a great extent because of its dynamic metabolite CCl_3 (this metabolite can extract hydrogen from unsaturated fats, starting the lipid peroxidation), lead to cell injury, lastly liver harm. Additionally, another scientist [38] expressed that the adequacy of any hepatoprotective medication is subject to its ability of either diminishing the hurtful impact or reestablishing the ordinary hepatic physiology that has been dispersed by a hepatotoxin. In this association, the current investigation uncovered that *M. indica* (*Tommy Atkins*) peels methanolic extract decreased ($p < 0.05$) CCl_4 prompted raised catalyst levels in tried groups, showing the assurance of primary uprightness of hepatocytic cell film or recovery of harmed liver cells. As recently noted, and like the outcomes accomplished for different plants in the writing [39-40] our perceptions and discoveries can be ascribed to the cell reinforcement elements *M. indica* (*Tommy Atkins*) peels extract that most likely repress lipid peroxidation and thusly restraint of oxidative pressure. Subsequently, the cell films stay unblemished and thus cells are forestalled to enter the corruption step.

Histopathological Results

Liver:

Minuscule assessments of areas of liver from ordinary control rats show the typical engineering of hepatic lobules. The focal veins lies at the focal point of the lobules encompassed by ropes of hepatocytes. Between the strands of hepatocytes, the hepatic sinusoids are seen (Figure 2). Histopathological examination of liver from rats regulated with CCl_4 alone demonstrating Histopathological examination of liver from rats regulated with CCl_4 alone demonstrating interruption of the liver tissue with loss of lobular plan, spanning fibrosis with collagenous septa development extended gateway lot to focal vein with mononuclear cells, vacuolar degeneration, and corruption of hepatocytes (Figure 3).

Liver segments of rats directed with CCl_4 and *M. indica* (*Tommy Atkins*) peels methanolic extract demonstrating gentle incendiary cells invasions around focal vein, vacuolar degeneration, and putrefaction of hepatocytes. Binucleated and actuated Kupffer cells were seen (Fig. 4). Microscopic examinations of sections of liver from normal control rats show the commonplace designing of hepatic lobules.

The central veins lies at the point of convergence of the lobules enveloped by ropes of hepatocytes. Between the strands of hepatocytes, the hepatic sinusoids are seen (Fig. 2). Histopathological assessment of liver from rats managed with CCl_4 alone exhibiting interference of the liver tissue with loss of lobular arrangement, spreading over fibrosis with collagenous septa advancement stretched out passage parcel to central vein with mononuclear cells, vacuolar degeneration, and debasement of hepatocytes (Fig. 3). Liver segments of rats treated with CCl_4 and *M. indica* (*Tommy Atkins*) peels methanolic extract exhibiting delicate irritable cells attacks around central vein, vacuolar degeneration, and degeneration of hepatocytes as well as binucleated and activated Kupffer cells were seen (Fig. 4).

Microscopic examinations of sections of liver from normal control rats show the commonplace designing of hepatic lobules.

In the current investigation, the biochemical discoveries were likewise affirmed by histopathological perceptions. The progressions generally incorporate hepatocellular putrefaction or apoptosis, greasy group, provocative cells invasion and other histological appearances which were likewise reliable with the discoveries of different creators [41].

Table (1): Effect of oral administration of CCl_4 alone or with *M. indica* (*Tommy Atkins*) peels methanol extract, on some biochemical parameters of male albino rats

Parameters	Experimental groups		
	Control	CCl_4	*M. indica* (*Tommy Atkins*) peels MeOH extract + CCl_4
TL (mgdL^{-1})	512.04 ± 43.06	663.6 ± 42.3	611.2 ± 39.3
TC (mgdL^{-1})	118.20 ± 12.97	230.8 ± 20.3	175.7 ± 14.6
TG (mgdL^{-1})	104.40 ± 7.34	171.8 ± 14.5	132.6 ± 12.3
LDL-C (mgdL^{-1})	61.20 ± 9.87	161.4 ± 16.7	123.7 ± 11.5
HDL-C (mgdL^{-1})	36.60 ± 6.40	28.6 ± 3.5	31.2 ± 5.8
TP (g d L^{-1})	6.68 ± 0.22	6.27 ± 0.3	6.42 ± 0.9
A (g d L^{-1})	4.42 ± 0.13	3.2 ± 0.2	4.1 ± 0.09
G (g d L^{-1})	2.46 ± 0.24	3.9 ± 0.1	3.2 ± 0.1
A/G ratio	1.72 ± 0.16	0.95 ± 0.07	1.4 ± 0.07
ASAT (U L^{-1})	33.02 ± 1.30	109.3 ± 25.9	89.5 ± 11.3
ALAT (U L^{-1})	25.60 ± 1.50	69.6 ± 9.7	50.6 ± 7.1
ALP (U L^{-1})	55.30 ± 3.84	79.2 ± 11.8	68.5 ± 8.4
TBil (mg d L^{-1})	0.66±0.02	0.94 ± 0.05	0.79 ± 0.06
DBil (mg d L^{-1})	0.11 ± 0.005	0.15 ± 0.009	0.12 ± 0.003

Data are represented as mean ± standard error.

Table (2): Effect of oral administration of CCL_4 alone or with *M. indica* (*Tommy Atkins*) peels methanolic extract, on some antioxidants of male albino rats

Parameters	Experimental groups		
	Control	CCl_4	*M. indica* (*Tommy Atkins*) peels MeOH extract + CCl_4
MDA (nmol g^{-1} liver)	4.48 ± 0.11	9.8 ± 0.6	6.7 ± 0.3
GSH (mg g^{-1} liver)	40.04 ± 5.10	21.7 ± 7.8	33.5 ± 8.9
CAT (U g^{-1} liver)	104.3 ± 17.1	45.4 ± 9.7	85.2 ± 18.6
SOD (U g^{-1} liver)	9.56 ± 0.17	5.6 ± 0.9	7.3 ± 0.4
GR (U g^{-1} liver)	73.20 ± 2.71	34.8 ± 2.8	59.1 ± 3.4

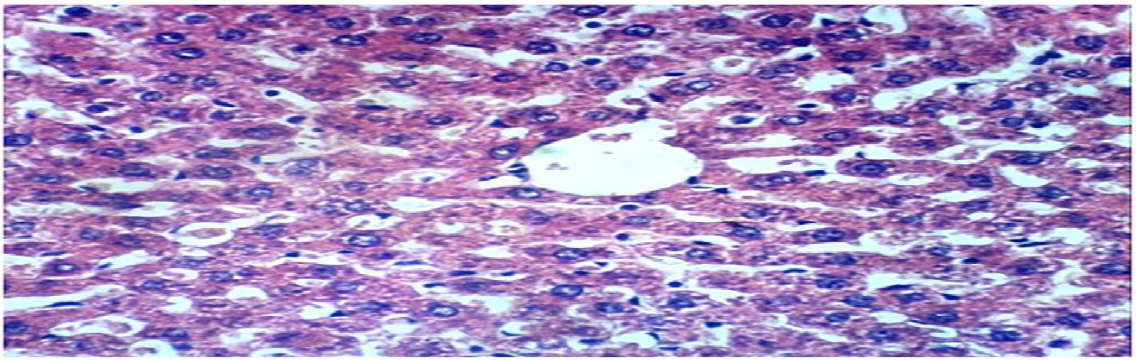


Figure 2: Photomicrograph of section in liver of control rat shows normal histological structure of hepatic lobules central vein, hepatocytes, blood sinusoids, and nuclei (H&E, ×400).

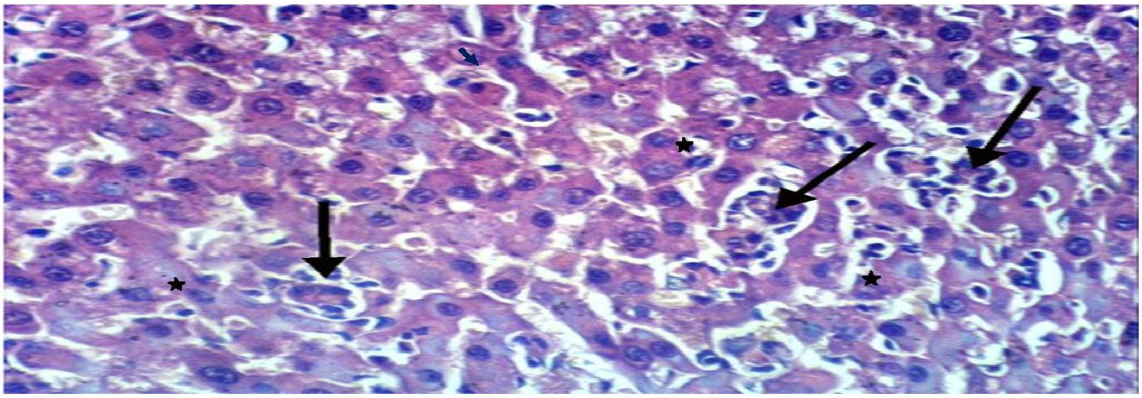


Figure 3: Photomicrograph of section in liver of rat administered with CCl_4 alone showing disruption of the liver tissue with loss of lobular arrangement, bridging fibrosis with collagenous septa formation expanded portal tract to central vein (arrow) with mononuclear cells, vacuolar degeneration, and necrosis of hepatocytes (star). Dilated and congested central vein was observed (arrowhead) and pyknotic nuclei (H&E, ×400).

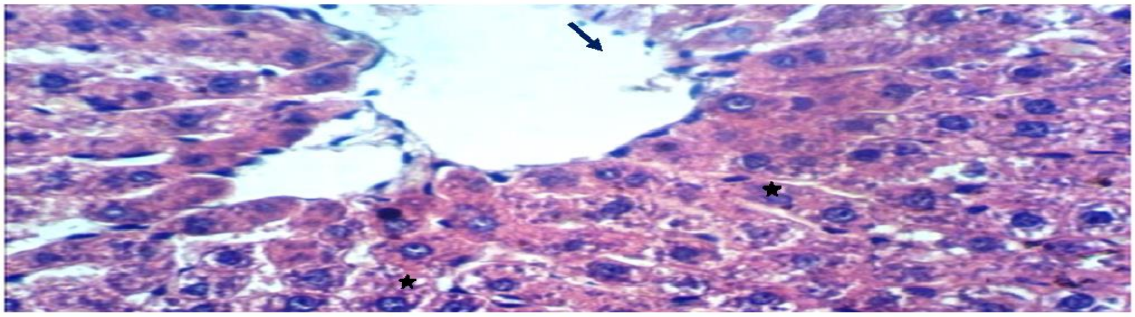


Figure 4: Photomicrograph of section in liver of rat administered with CCl_4 and *M. indica* (*Tommy Atkins*) peels methanol extract showing mild inflammatory cells infiltrations around central vein (arrow), vacuolar degeneration, and necrosis of hepatocytes (star). Binucleated and activated Kupffer cells were noticed (H&E, ×400).

Conclusion

The present study has revealed that *M. indica* (*Tommy Atkins*) peels are capable of synthesizing and accumulating different types of phenolics, including mainly ellagitannins, gallotannins which are elucidated for the first time from this plant. The isolation for gallotannins that could be useful for the preparation of these compounds either as a crude fraction or single pure sample from the peels and rich gallotannins plant extracts.

According to these results, it could be concluded that the peels extract can be a potential source of an attractive candidate for ameliorating of hepatotoxicity induced by CCl_4 through scavenging free radicals, improved liver functions, and normalizing the liver histopathological architecture.

References:

[1] Barreto, J. C., Trevisan, M. T., Hull, W. E., Erben, G., De Brito, E. S., Pfundstein, B. and Owen, R. W. (2008). Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peels of mango (*Mangifera indica* L.). Journal of agricultural and food chemistry, 56(14), 5599-5610.

[2] Berardini, N., Fezer, R., Conrad, J., Beifuss, U., Carle, R. and Schieber, A. (2005). Screening of mango (*Mangifera indica* L.)cultivars for their contents of flavonol *O*-and xanthone C-glycosides, anthocyanins, and pectin.Journal of agricultural and food chemistry,53(5),1563-1570

[3] Nayan, V., Onteru, S. K. and Singh, D. (2018). *Mangifera indica* flower extract mediated biogenic green gold nanoparticles: Efficient nano catalyst for reduction of 4- nitrophenol. Environmental Progress & Sustainable Energy, 37(1), 283-294.

[4] Ma, X., Wu, H., Liu, L., Yao, Q., Wang, S., Zhan, R. and Zhou, Y. (2011). Polyphenolic compounds and antioxidant properties in mango fruits. *Scientia Horticulturae*, 129(1),102-107.

[5] Masibo, M. and He, Q. (2009). Mango bioactive compounds and related nutraceutical properties a review. Food Reviews International, 25(4), 346-370.

[6] Arts, I. C., van de Putte, B. and Hollman, P. C. (2000). Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *Journal of agricultural and food chemistry*, 48(5), 1746-1751.

[7] Mukherjee, S. K. (1972). Origin of mango (*Mangifera indica*). *Economic Botany*, *26*(3), 260-264.

[8] García-Rivera, D., Delgado, R., Bougarne, N., Haegeman, G. and Berghe, W. V. (2011). Gallic acid indanone and mangiferin xanthone are strong determinants of immunosuppressive anti-tumour effects of *Mangifera indica* L. bark in MDA-MB231 breast cancer cells. Cancer Letters, 305(1), 21-31.

[9] Schieber, A., Berardini, N., and Carle, R. (2003). Identification of flavonol and xanthone glycosides from mango (*Mangifera indica* L. Cv.“*Tommy Atkins*”) peels by high-performance liquid chromatography-electrospray ionization mass spectrometry. Journal of Agricultural and Food Chemistry, 51(17), 5006-5011.

[10] Luo, F., Fu, Y., Xiang, Y., Yan, S., Hu, G., Huang and Chen, K. (2014). Identification and quantification of gallotannins in mango (*Mangifera indica* L.) kernel and peels and their antiproliferative activities. journal of functional foods, 8, 282-291.

[11] Andersen Q.M. and Markham K.R. (2006). In "Flavonoids: Chemistry, Biochemistry and Applications". Boca Raton, FL: CRC., press, pp: 53–82.

[12] El-Hawary, S. S. and Rabeh, M. A. (2014). *Mangifera indica* peels: A common waste product with impressive immunostimulant, anticancer and antimicrobial potency. Journal of Natural Sciences Research www. iiste. org, ISSN, 2224-3186.

[13] González-Sarrías, A., Yuan, T. and Seeram, N. P. (2012). Cytotoxicity and structure activity relationship studies of maplexins A–I, gallotannins from red maple (Acer rubrum). Food and Chemical Toxicology, 50(5), 1369-1376.

[14] Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by Thio barbituric acid reaction. Analytical biochemistry, 95(2), 351-358.

[15] Ohkawa, H., N. Ohishi and K. Yagi. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

[16] Beutler, E., O. Duron and B.M. Kelly, (1963). Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.

[17] Aebi, H. (1984). Catalase in vitro; Methods Enzymol, 105: 121-126.

[18] Nishikimi, M., Rao, N. A. and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and biophysical research communications, 46(2), 849-854.

[19] Goldberg, D.M. and R.J. Spooner (1983). "Method for the determination of glutathione reductase; Methods Enzymatic Anal", 3: 258-265.

[20] Nawwar M.A., Hussein S.A. and Merfort I. (1994). NMR spectral analysis of polyphenols from *Punica granatum*. Phytochemistry, 36 (3): 793-798.

[21] Sánchez, E., Heredia, N., Camacho- Corona, M. D. R. and García, S. (2013). Isolation, characterization and mode of antimicrobial action against Vibrio cholerae of methyl gallate isolated from *Acacia farnesiana*. Journal of applied microbiology, 115(6), 1307-1316.

[22] Hussein, S. A., Barakat, H. H., Merfort, I. and Nawwar, M. A. (1997). Tannins from the leaves of *Punica granatum*. Phytochemistry, 45(4), 819-823.

[23] Lee, E. J., Kim, J. S., Kim, H. P., Lee, J. H. and Kang, S. S. (2010). Phenolic constituents from the flower buds of *Lonicera japonica* and their 5-lipoxygenase inhibitory activities. Food chemistry, 120(1), 134-139.

[24] Berregi, I., Santos, J. I., del Campo, G., Miranda, J. I. and Aizpurua, J. M. (2003). Quantitation determination of chlorogenic acid in cider apple juices by 1H-NMR spectrometry. Analytica Chimica Acta, 486(2), 269-274.

[25] Sahar, A.M. Hussein, Amani M.D. El-Mesallamy, Salma O.K. Othman, Abd El-Mohsen M. Soliman. (2020). Identification of Novel Polyphenolic Secondary Metabolites from *Pistacia Atlantica* Desf.

and Demonstration of their Cytotoxicity and CCl4 induced Hepatotoxicity in Rat. Egyptian Journal of Chemistry,63(12),117-130

[26] Chien-Chang Shen (1993) "Nuclear magnetic resonance studies of 5, 7 dihydroxy flavonoids", Phytochemistry, 34, (3), 843.

[27] Al-Fartosi KG, Majid A, Auda MA (2012). The role of Camel's milk against some oxidant-antioxidant markers of male rats treated with CCl_4. Int J Res Pharmaceut Biomed Sci 3 (1):385-389.

[28] Sreepriya M, Devaki T and Nayeem M. (2001) Protective effects of Indigofera tinctoria L. against D-Galactosamine and carbon tetrachloride challenge on 'in situ' perfused rat liver. Indian J Physiol Pharmacol 45: 428-434.

[29] Khan AA and Alzohairy M. (2011). Hepatoprotective effects of camel milk against CCl_4-induced hepatotoxicity in Rats. Asian J Bio chem 6(2):171-180.

[30] Arıcı OF and Cetin N. (2011). Protective role of ghrelin against carbon tetrachloride (CCl_4 induced coagulation disturbances in rats. Regul Pept 166: 139-142.

[31] Pierson JT, Monteith GR, et al. (2014). Phytochemical extraction, characterisation and comparative distribution across four mango (Mangifera indica L.) fruit varieties. Food Chem. 15: 149:253-63.

[32] Park WH, Lee SK and Kim CH, A. (2005). Korean herbal medicine, panax notoginseng, prevents liver fibrosis and hepatic microvascular dysfunction in rats. Life Sci 76:1675–1690.

[33] Palanive MG, Rajkapoor B and Kumar RS. (2008) Hepatoprotective and antioxidant effect of pisonia aculeata L. Against CCl_4- induced hepatic damage in rats. Sci Pharm 76:203–215.

[34] Yousef MI, Saad AA and El-Shennawy LK. (2009). Protective effect of grape seed proanthocyanidin extract against oxidative stress induced by cisplatin in rats. Food Chem Toxicol. 47:1176- 83.

[35] Mirza B., Croley C. R., Ahmed M. et al. (2020) Mango (*Mangifera indica* L.): a magnificent plant with cancer preventive and anticancer therapeutic potential, Crit Rev Food Sci Nutr. 8:1-27.

[36] Ediriweera MK, Tennekoon KH and Samarakoon SR. (2017). A Review on Ethnopharmacological Applications, Pharmacological Activities, and Bioactive Compounds of Mangifera indica (Mango). Evid Based Complement Alternat Med. 2017: 1-24.

[37] Umamahesh K, Gandhi AD and Reddy OVS. (2020). Ethnopharmacological Applications of Mango (Mangiferaindica L.) Peels - A Review. Curr Pharm Biotechnol.;21(13):1298-1303

[38] Rafiee f, Heidari r, Ashraf h. and Rafiee p. (2013). Protective effect of Berberis integerrima fruit extract on carbon-tetrachlorideinduced hepatotoxicity in rats. J Fasa Univ Med Sci 3:179-87.

[39] Arampath PC, Dekker M. (2019). Bulk storage of mango (*Mangifera indica* L.) and pineapple (Ananas comosus L.) pulp: effect of pulping and storage temperature on phytochemicals and antioxidant activity. J Sci Food Agric. 99(11):5157-5167.

[40] Mwaurah PW, Kumar S, Kumar N, et al. (2020). Physicochemical characteristics, bioactive compounds and industrial applications of mango kernel and its products: A review. Compr Rev Food Sci Food Saf. 19(5):2421-2446.

[41] Kumar M, Saurabh V, Tomar M, et al. (2021). Mango (*Mangifera indica* L.) Leaves: Nutritional Composition, Phytochemical Profile, and Health-Promoting Bioactivities. Antioxidants (Basel). 10(2):299.