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Chemical Constituents and Biological Efficacy Evaluation of *Traganum Nudatum* Aerial Parts

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Abstract

The aim of this study focused to investigate the chemical constituents of chloroform and methanol extracts of *Traganum nudatum* aerial parts and evaluation of their related biological ability as antitumor, anti-diabetic and anti-inflammatory activity. The GC-Ms analysis of chloroform extract revealed the identification of 45 compounds (26 terpenoids, 13 fatty acids methyl ester, 3 sterols, 2 alkaloids, 1 ally benzene). Carvacrol and citronellol were the major components of the terpenoid compounds while elaidic and stearic acids were to fatty acid methyl ester. The methanol extract was applied to polyamide column chromatography which led to isolation and identification of eight phenolic compounds. The chemical structure of the isolated compounds was identified by the physical and chemical properties using UV, MS, ^{1}H and ^{13}C-NMR as hesperidin **1** isorhamentin-3-*O*-α-robinobioside **2**, caffeic acid **3**, isorhamentin **4**, *p*-coumaric **5**, sinapic acid **6**, 7-hydroxy flavone **7** and quinic acid **8**. The evaluation of the biological activity showed that the chloroform extract has antitumor and antidiabetic effect better than the methanol extract. While the methanol extract exhibited anti-inflammatory inhibition efficacy more than the chloroform extract that showed no anti-inflammatory activity.

Keywords: *Traganum Nudatum*; chemical constituents; antitumor; anti-diabetic; anti-inflammatory

1. Introduction

One of the greatest blessings of the Creator on us is that created a drug for every ailment, this drug may be next to our eyes, but we do not know . This drug is a phytotherapy medicine. Therefore, after the tremendous scientific revolution in the development of the pharmaceutical industries, which depend on the extraction of therapeutic materials from medical and natural therapeutic sources. The world tends to concern the present and new researches with the activity of the medicinal plants as remedy ailments which, characterized by their activity, few side effects, cheap and easy availability. T. nudatum is a member of family amarnthaceae which is a native halophytic shrub in arid zones of the Mediterranean basin. It grows in high temperatures and can tolerate increased salinity and aridity [1]. In folk medicine, the leaves of T. nudatum were used to treat wounds, rheumatism, diarrhea, dermatoses [2], treat diabetes and various pains [3], also as a remedy for gastric problems, pruritus and pimples [4] and usefulness as protective agents against oxidative DNA damage [5]. Cancer is a life-threatening disease that kills more than 7.6 million people annually, including many types including breast, colon, hepatic, prostate cancer..... etc. [6]. Till now, the known type treatment for different cancer types is resection, radiation, and chemotherapy, which led to many harmful and killing effects for the normal cells besides the infected cells. so, the discovery use of natural products alongside or instead of chemotherapy can give promising results. According to WHO, it was estimated that 3% of the world's population suffered from diabetes and the prevalence is expected to double by the year 2025 to 6.3% [7]. Diabetes mellitus is a chronic disease characterized by raised blood glucose levels, and made disturbances in carbohydrate, protein, and fat metabolism [8]. The medical community is still grappling with how to treat diabetes without causing side effects. Therefore, The significance of natural therapies comes to compete with this challenge. The Inflammation is the protective response of the

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organisms to eliminate harmful stimuli and begin the healing process. It has also been linked to the development of a variety of cancers. An effective anti-inflammatory medicine should be able to prevent inflammation from developing while maintaining normal homeostasis [9]. Nowadays, several herbal medications play an important role in the inhibition of inflammatory. As we interest with the evaluation of biological and chemical properties of the medicinal plants and discovering new alternative herbal medicine with more activity, low cost and no side effects utilizing as a promising remedy for more diseases [10-14]. In view of this, and in continuation of our studies on the medicinal phytotherapy on halophyte amarnthaceae plants [15], the objective of this work was the isolation and identification of the chemical compositions of halophyte *T. nudatum* aerial parts, and investigation of its biological efficacy as antitumor, antidiabetic and anti-inflammatory activity.

Material and methods

Plant material

Aerial parts of *T. nudatum* (Family: Amarnthaceae) were collected from their wild habitat in Matrouh City, western desert of Egypt in June 2019. The plant samples were identified and authenticated by Dr. Omran Ghaly, Desert Research Center. A voucher herbarium sample was deposited in the herbarium of Desert Research Center (CAIH) with Code Number: CAIH-1023-R.

Chemicals and reagents

All chemicals and reagents used in this study were of high quality and analytical grade purchased from Sigma-Aldrich.

Chemical studies

Extraction

The air-dried powdered of the *T. nudatum* (500 g) were extracted successively by soxhlet apparatus beginning with *n*-hexane, chloroform and finally methanol for 48 h till exhaustion for each solvent. Each extract was concentrated under vacuum to yield semisolid extracts (13g, 3.5g, 7.2g), respectively. The chloroform extract was subject to GC-Ms analysis. While, the methanol extract components was isolated using polyamide column chromatography. The chemical structure of the isolated compounds was identified by the physical and chemical properties using UV, MS, ^{1}H and ^{13}C-NMR.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of the chloroform extract was performed at the Regional Center for Mycology and Biotechnology (RCMB) at Al- Azhar University, on Thermo Scientific Trace 1310 Gas Chromatograph attached with ISQ LT single quadrupole mass spectrometer, operating in the EI mode at 70 eV, equipped with a split/splitless injector (200°C). Helium was used as carrier gas (1 mL/min) and the capillary columns used were an DP5-MS (30 m x 0.25 mm; film thickness 0.25 mm). The transfer line temperature was kept at 290 °C and 300 °C respectively with electron multiplier voltage of 1 Kv. Identification and interpretation of phytoconstituents on mass-spectrum GC-MS was conducted using the reference library of the National Institute of Standards and Technology (NIST), US, along with Willey 5 and mass finder. The constituent percentages were measured based on the peak area [16].

Isolation of Compounds:

The MeOH extract (7.2 g) was subjected to polyamide column (5x150 cm) with eluting system H_2O/MeOH with decreasing polarity till reach 100 % MeOH which afforded 48 fractions (M1-M48). All fractions were applied to 1 MM paper chromatography (PC) run in (BAW) (4:1:5) and (15% AcOH). All similar fractions were collected together and then were individually separated with other sub-column, TLC and PC. The final isolated compounds were purified by Sephadex column (1.5x50 cm) to obtain pure compounds. Compounds **1, 2** and **3** were isolated from collected fractions (M4-M18) with further polyamide sub-column isolation which afforded 13 sub-fractions (MA1-MA13). Similar sub-fractions (MA1-MA8) were collected together and applied to PPC with an eluting system (EtOAc: MeOH: H_2O) (18:13: 2.5) which gave two major spots, with further purification by sephadex column give two compounds 1 and 2. Similar sub-fractions (MA10-MA13) were collected then applied to PTLC with an eluting system ($CHCl_3$: EtOAc: MeOH) (30: 5: 4) to give compound 3. Fractions (M20-M23) were collected then applied to PTLC with the solvent system (toluene: EtOAc: Formic acid) (5: 4: 1) to give compound 4. Fractions (M26-M33) were collected then applied to sup-column polyamide (MeOH: H2O) (90:10) to afford 8 sub-fractions (MB1-MB8). (MB1-MB6) were collected then applied to PTLC ($CHCl_3$: EtOAc: MeOH) (18: 7.5: 3) to give compounds **5** and **6**. Fractions (M35-M45) were collected and applied to PPC (B: A: W) (3:1:1 upper layer) to give compounds **7** and **8**.

Biological evaluation

Antitumor activity

The mammalian cell lines: HepG-2 cells (human hepatocellular cancer cell line, HCT-116 (human colon carcinoma) and PC-3 cells (human prostate cancer cell line) were gotten from the American Type Culture Collection (ATCC, Rockville, MD). The tumor cell lines were conserved by serial sub-culturing in the National Cancer Institute, Cairo, Egypt. The cytotoxic effect of chloroform and MeOH extracts of *T. nudatum* aerial parts were evaluated in the National Cancer Institute, Cairo University according to the MTT assay method described in [17].

Antidiabetic activity

α- Amylase inhibition method

The α- amylase inhibitory activity of chloroform and MeOH extracts of *T. nudatum* were carried out according to the method described in [18,19]. Briefly, The enzyme solution was prepared by dissolving α-amylase in 20mM phosphate buffer (6.9) at the concentration of 0.5 mg/ml. 1ml of each extract of various concentrations (7.81-1000µg/ml) and 1ml of enzyme solution were mixed together and incubated at 25 °C for 10 min. After incubation, 1ml of starch (0.5%) solution was added to the mixture and further incubated at 25 °C for 10 min. The reaction was then stopped by adding 2 ml of dinitrosalicylic acid (DNS, color reagent) heating the reaction mixture in a boiling water bath (5 min). The absorbance was colorimetrically measured at 565 nm after cooling. % inhibition calculated from formula (1-As/Ac) ×100, where Ac control is the absorbance of the control reaction (which contains all reagents except the test sample) and as sample is the absorbance of the test sample. Acarbose drug was employed as a standard reference drug. The IC_{50} value was defined as the concentration of α-amylase inhibitor to inhibit 50% of its activity under the assay conditions.

α-glucosidase inhibitory activity

α-glucosidase inhibitory activity of chloroform and MeOH extracts of *T. nudatum* were carried out according to the standard method with minor modification [20]. In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH=6.8), 10 µl α -glucosidase (1 U/ml), and 20 µl of changeable concentrations of extracts (7.81 to 1000 μg/mL) was pre-incubated at 37°C for 15 min. Then, 20 µl 4-Nitrophenyl-β-D-glucoside (P-NPG) (5 mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was halted by addition of 50 µl Na_2CO_3 (0.1 M). By using Multiplate Reader, the absorbance of the liberated *p*-nitrophenol was measured at 405 nm. Acarbose was used as a standard drug, with concentrations ranging from 7.81 to 1000 µg/mL). A control experiment was set up in parallel without the test substance, and each experiment was repeated three times. The results were computed using the formula Inhibitory % = (1-As/Ac)×100, where As is the absorbance in the presence of the test drug and Ac is the absorbance in the absence of the test substance.

Anti-inflammatory activity:

Membrane stabilization

The anti-inflammatory activity of the chloroform and methanol extracts of *T. nudatum* was evaluated by *in vitro* HRBC membrane stabilization method. Briefly, Whole blood was taken from rats through heart puncture using heparinized syringes. The blood was cleaned three times with isotonic buffered solution (154 mM NaCl) in a 10 mM Na_3PO_4 buffered (pH 7.4). The blood was centrifuged for 10 minutes at 3000 g. Hypotonic solution-induced erythrocyte hemolysis was used to evaluate of membrane stabilizing activity of the two extracts. The test sample contained on stock erythrocyte (RBCs) suspension (0.50 ml) was mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM Na_3PO_4 buffered saline (pH 7.4), which contained the extract (0.98-125 µg/ml) or indomethacin. The absorbance of the supernatant was measured at 540 nm in 96 well plates. According to a modified method described in [21].

Cyclooxygenase (COX 2) inhibition assay:

The samples at the concentration range of 0.98-125 µg /ml of the chloroform and methanol extracts of *T. nudatum* were tested in order to investigate the anti-inflammatory response by inhibiting the COX-2 enzyme. The COX activity was monitored, as the result of the N,N,N,N-tetramethyl-*p*-phenylenediamine (TMPD) oxidation reaction with arachidonic acid. This assay was performed according to method mentioned in [22] with slight modifications. The inhibitory activity was determined by monitoring the absorbance's increase at 611 nm using a microplate reader (BIOTEK; USA). The inhibitory percentages were calculated according to the formula: Inhibitory activity (%) = (1–As /Ac) ×100, where, As is the absorbance in the presence of test substance and Ac is the absorbance of control.

Results and Discussions

Chemical studies:

GC-Ms analysis of chloroform extract:

The GC-Ms analysis of chloroform extract of *T. nudatum* aerial parts led to the identification and qualification of forty-five components accounting for 99.99% of the total components outlined in (Table 1). The GC-Ms analysis of chloroform extract revealed to the identification of 45 different compounds (26

terpenoids, 13 fatty acid methyl ester, 3 sterols, 2 alkaloids, 1 ally benzene). Carvacrol and citronellol were the major components of terpenoid compounds while, Elaidic, Stearic acids were the highest concentration of fatty acid methyl ester. On the other side, Nizatidine and Stigmasterol represented the high percent for alkaloid and sterol components respectively. The high current percent of these components may be related to its high lipodial content of the halophyte *T. nudatum* plant which formed as a result of its adaptation to growth conditions in its natural habitat. All chemical structures of the highest area % identified compounds were illustrated in the table (1) and figure (1).

Table (1): GC-Ms Chemical composition of the chloroform extract of *T. nudatum* aerial parts

RT	Compound Name	Area %	M. F.	M.Wt	Type
12.75	Citronellol	7.04	$C_{10}H_{20}O$	156	Terpinoids, dervatives
13.52	Geraniol	5.69	$C_{10}H_{18}O$	154	
13.86	Citronellyl formate	2.23	$C_{11}H_{20}O_2$	184	
14.53	Geranyl formate	1.67	$C_{11}H_{18}O_2$	182	
14.87	Carvacerol	8.91	$C_{10}H_{14}O$	150	
15.71	α- copaene	0.99	$C_{15}H_{24}$	204	sesquiterpene
16.13	Eugenol	4.10	$C_{10}H_{12}O_2$	164	allylbenzene
17.61	Caryophyllene	1.55	$C_{15}H_{24}$	204	sesquiterpene
18.24	Citronellyl propionate	1.58	$C_{13}H_{24}O_2$	212	Monoterpenoid, dervatives
18.44	Humulene	0.61	$C_{15}H_{24}$	204	
19.04	Lavandulyl propanoate	1.92	$C_{13}H_{22}O_2$	210	sesquiterpenes
19.19	Germacrene D	1.84	$C_{15}H_{24}$	204	
19.51	Cubebol	0.89	$C_{15}H_{26}O$	222	
19.78	Nizatidine	2.53	$C_{12}H_{21}N_5O_2S_2$	331	Thioalkaloids
19.95	Nerolidol	0.77	$C_{15}H_{26}O$	222	Sesquiterpene, deravitives
20.19	Cadina-3,9-diene	1.12	$C_{15}H_{24}$	204	
20.54	Caryophyllene oxide	1.59	$C_{15}H_{24}O$	220	
20.94	Butanoic acid, 3,7-dimethyl-2,6-octadienyl ester	3.41	$C_{14}H_{24}O_2$	208	Fatty acid ester
21.21	(-)-Globulol	0.64	$C_{15}H_{26}O$	222	Sesquiterpenoid
21.31	(-)-Spathulenol	1.37	$C_{15}H_{24}O$	220	
21.68	Viridiflorol	0.41	$C_{15}H_{26}O$	222	
21.98	Ledol	2.36	$C_{15}H_{26}O$	222	
22.17	β-Eudesmol	4.59	$C_{15}H_{26}O$	222	
22.63	α-Guaiene	1.33	$C_{15}H_{24}$	204	
22.89	Cubenol	1.76	$C_{15}H_{26}O$	222	
23.00	Aristolene	0.42	$C_{15}H_{24}$	204	
23.17	Hedycaryol	1.28	$C_{15}H_{26}O$	222	
23.37	Citronellyl tiglate	1.07	$C_{15}H_{26}O_2$	238	
24.43	Stigmasterol	2.09	$C_{29}H_{48}O$	412	Steroids
24.62	B-Sitosterol	0.91	$C_{29}H_{50}O$	414	
23.96	Cholestan-3-ol, 2-methylene (3á,5à)	0.71	$C_{28}H_{48}O$	400	
24.71	Hexanoic acid,3,7-dimethyl -2,6-octadienyl ester	1.29	$C_{16}H_{28}O_2$	252	Fatty acid ester
24.86	Tetradecanoic acid (Myristic acid)	0.51	$C_{14}H_{28}O_2$	228	
25.47	Hexanoic acid, 3,7-dimethyl-2,6-	1.66	$C_{16}H_{28}O_2$	252	

	octadienyl ester, (Z)-				
26.42	1,3,5-triazine-2,4-diamine, 6-chloro-n-ethyl	0.54	$C_{14}H_{18}ClN_5$	291	Alkaloids
27.51	Heptanoic acid,3,7-dimethyl-2,6-octadienyl ester,(E)-	2.75	$C_{17}H_{30}O_2$	266	
28.02	17-Octadecynoic acid	1.42	$C_{18}H_{32}O_2$,	280	
29.05	Hexadecanoic acid, methyl ester	1.82	$C_{17}H_{34}O_2$	270	
30.96	Margaric acid methyl ester	1.48	$C_{18}H_{36}O_2$	284	
32.23	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (Linoleic acid, methyl ester)	2.80	$C_{19}H_{34}O_2$	294	Fatty acid ester
32.34	9-Octadecenoic acid, methyl ester, (E)- (Elaidic acid, methyl ester)	9.73	$C_{19}H_{36}O_2$,	296	
32.36	Oleic acid, methyl ester	1.86	$C_{19}H_{36}O_2$	296	
33.56	11-Octadecenoic acid, (Z)- (Cis-Vaccenic acid)	2.73	$C_{18}H_{34}O_2$	282	
36.67	Stearic acid, methyl ester	3.56	$C_{19}H_{38}O_2$	298	
37.50	Cedran-8-ol	0.46	$C_{15}H_{26}O$	222	Sesquiterpene alcohol

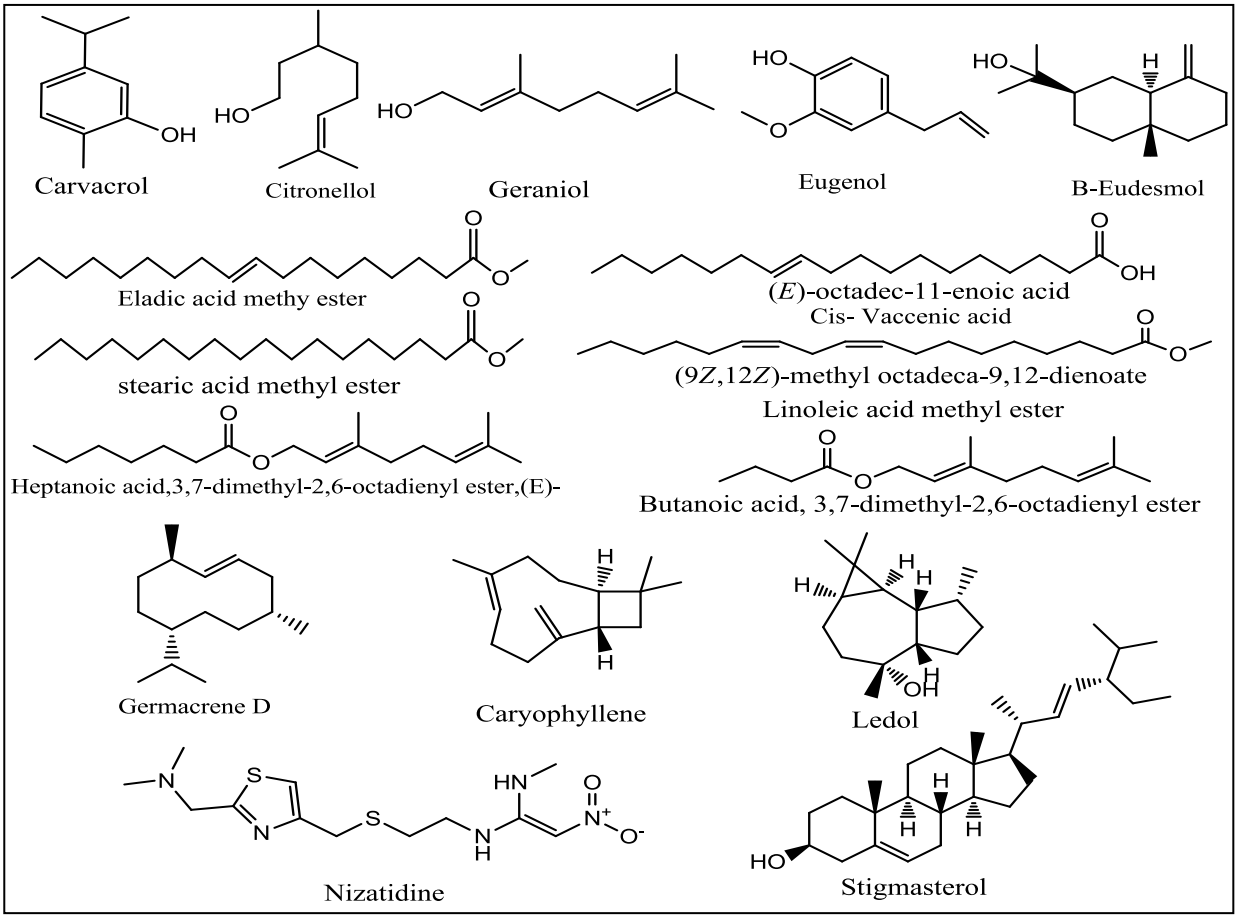

Fig (2): Chemical structure of identified highest concentration compounds by GC-Ms analysis of chloroform extract of *T. nudatum* aerial parts

Isolation of Compounds

Isolation of Compounds

The isolation of phenolic and flavonoid compounds from methanol extract by different chromatographic methods (CC, TLC and PC) led to the isolation and identification of eight compounds (4 phenolic acids and 4 flavonoids, two of them were flavonoid glycosides). The isolated compounds were identified as hesperidin **1**, isorhamnetin-3-*O*-α-robinobioside **2**, caffeic acid **3**, isorhamentin **4**, *p*-coumaric **5**, sinapic acid **6,** 7-hydroxy flavone **7** and quinic acid **8**. The structure elucidation of these compounds was established based on chemical and spectral (UV, MS,^{1}H and ^{13}C-NMR spectroscopy). As well, the confirmation of these compounds was performed by comparison obtained data with those available in the literature. The physical and spectral data of isolated compounds **1, 3, 5** were in accordance with those reported in our previous study in the literature [13].

Identification of compound isorhamentin 3-*O*-*α* – L-rhamnopyranoside (1- 6)-*β*- D-galactose, isorhamentin-3-*O*-*α*-robinobioside

It was isolated as yellow crystalline which showed deep purple color under UV light converted to yellow when exposure to NH_3 vapour. it was found to exhibit *Mr* of 624 in ESI-MS analysis ([M+H]- at *m/z*=625). Rf 0.48 (BAW), 0.64 (15% AcOH). UV λ_{max} (nm): MeOH: 253, 354; (NaOMe): 270, 328, 416; (NaOAc): 270, 321; (NaOAc/H_3BO_3): 253, 360 nm. ^{1}H NMR (MeOD, 400 MHz) δ 7.71 ppm (1H, d, J=2.2 H- 2'), 7.45 (1H, dd, J=8.4, 2.2, H-6'), 6.87 (1H, d, J=8.4, H-5'), 6.34 (1H, d, J= 2.1, H-8), 6.09 (1H, d, J= 2.3, H-6), 5.67 (1H, d, J=6.5, H-1''), 4.57 (1H, d, J=2.5, H-1'''), 3.81 (3H, s, O-CH3), 3.2- 3.8 (10H complex and overlapped, m, CH-glycoside), 1.1 (3H, d, J=6.4, H-6'''). ^{13}C- NMR (MeOD, 400 MHz): δ 177.15 ppm (C-4), 166.697 (C-7), 161.779 (C-5), 157.90 (C-9), 156.23 (C-2), 151.87 (C-4'), 147.10 (C-3'), 134.481 (C-3), 123.55 (C-1'), 122.57 (C-6'), 115.65 (C-5'), 113.29 (C-2'), 105. 94 (C-10), 104.564 (C-1''), 102.43 (C-1'''), 99. 75 (C-6), 94. 77 (C-8), 78.07 (C-3''), 77. 34 (C-5''), 75.96 (C-2''), 73.815 (C-4''), 72. 36 (C-3'''), 71.31 (C-2'''), 70. 48 (C-4'''), 69.46 (C-5'''),68.87 (C-6''), 56.43 (OCH3), 17.81 (CH3 C-6'''). The chemical spectral data predicted the compound was to be isorhamnetin-3-*O*-robinobioside.

Identification of compound isorhamentin

It was isolated as faint yellow powder under UV light without any change with color when exposure to ammonia vapour which exhibited *Mr* of 316 in ESI-MS analysis. R_f 0.54 (BAW), 0.12 (6% AcOH). UV λ_{max} (nm): (MeOH) 251, 266, 372. ^{1}H NMR (MeOD, 400 MHz): δ 7.71 ppm (1H, d, J=2.2 H-2'), 7.45 (1H, dd, J=8.4, 2.2, H-6'), 6.87 (1H, d, J=8.4, H-5'), 6.34 (1H, d, J= 2.1, H-8), 6.09 (1H, d, J= 2.3, H-6), 5.67, 3.81 (3H, s, O-CH3). ^{13}C- NMR (MeOD, 400 MHz) δ 177.15 ppm (C-4), 166.697 (C-7), 161.779 (C-5), 157.90 (C-9), 156.23 (C-2), 151. 87 (C-4'), 147.10 (C-3'), 134.481 (C-3), 123.55 (C-1'), 122.57 (C-6'), 115.65 (C-5'), 113.29 (C-2'), 105. 94 (C-10), 99. 75 (C-6), 94. 77 (C-8), 56.43 (OCH_3). The chemical spectral data predicted the compound was to be isorhamentin.

Identification of compound sinapic acid

It was isolated as yellowish powder which exhibited *Mr* of 224 in ESI-MS analysis $[M+H]^+$ at *m/z* = 225. R_f 59 (BAW), 38 (6% AcOH); UV λ_{max} (nm): MeOH: 319, 290 sh., 230 sh . ^{1}H-NMR (MeOD): 6.27 (1H, d, *J* = 12.4 Hz, H-7 [α]), 6.82(1H, s, H-2), 6.82 (1H, s, H-6), 7.45(1H, d, *J* = 12.4 Hz, H-8 [β]), 3.81 (6H, s, -OCH_3). ^{13}C-NMR (MeOD): 172.4 (C=O), 116.5 (C-7), 148.9 (C-8), 126.7 (C-1), 108.3 (C-2, C-6)), 148.7 (C-3, C-5), 132.4 (C-4), 53.3 (OCH_3). The chemical spectral data predicted the compound was to be 3,5-dimethoxy-4-hydroxycinnamic acid.

Identification of compound 7-Hydoxy flavone

It was isolated as pale yellow powder under UV light without any changes when exposure to ammonia vapour and exhibited *Mr* = 238 in ESI-MS analysis $[M+H]^+$ at m/z = 239; UV λ_{max} (nm): MeOH: 253, 268, 309; (NaOMe): 265, 305, 355; (NaOAc): 264, 305, 355; (NaOAc/H_3BO_3): 253, 270,305 nm. ^{1}H-NMR (MeOD): 6.54 (1H, dd, *J*= 7.4, 2.1 Hz, H-6), 6.71 (1H, d, *J* =2.1 Hz, H-8), 6.95 (1H, s, H-3), 6.95 (1H, d, *J* =7.5 Hz, H-5), 6.88 (1H, d, *J*=8.4 Hz, H-5'), 7.67 (1H, dd, *J*= 2.2, 7.4 Hz, H-2'), 7.64 (1H, dd, *J*= 2.2, 7.4 Hz, H-6'), 7.51 (1H, m, H-3'), 7.45 (1H, m, H-4'), 7.51 (1H, m, H-5'). ^{13}C-NMR (MeOD): 163.1 (C-2), 104.2 (C-3), 179.8 (C-4), 125.6 (C-5), 115.1 (C-6), 164.3 (C-7), 106.7 (C-8), 157.5 (C-9), 113.1 (C-10), 130.7 (C-1'), 126.5 (C-2'), 128.5 (C-3'),129.0 (C-4'), 128.5(C-5'),126.5 (C-6'). The physical and chemical spectral data predicted to the chemical structure of compound was to be 7-Hydoxy flavone.

Identification of compound quinic acid

It was isolated as a white crystal powder which exhibited a *Mr* = 192 in ESI-MS; UV λ_{max} (nm): MeOH: 220; R_f BAW (0.46); ^{1}H-NMR (MeOD): 4.01 (1H, ddd, 9.4, 11.5, 4.5 Hz, H-3), 3.92 (1H, ddd, 9.4, 11.5, 4.5 Hz, H-5), 3.33 (1H, dd, 9.4, 3.4 Hz, H-4), 2.07 (1H, dd, *J* =2.1, 3.5 Hz, H-2_{eq}), 2.07 (1H, dd, *J* =2.1, 3.5 Hz, H-6_{eq}), 1.84 (1H, dd, *J*= 14.5, 3.7 Hz, H-2_{ax}), 1.84 (1H, dd, *J*= 14.5, 3.7 Hz, H-6_{ax}). ^{13}C-NMR (MeOD): 178.7 (C=O), 78.5 (C-1,

C-4), 36.5 (C-2, C-6), 70.4 (C-3, C-5). The obtained data is predicted to chemical structure of 1,3,4,5 tetrahydroxycyclohexane-1-carboxylic acid.

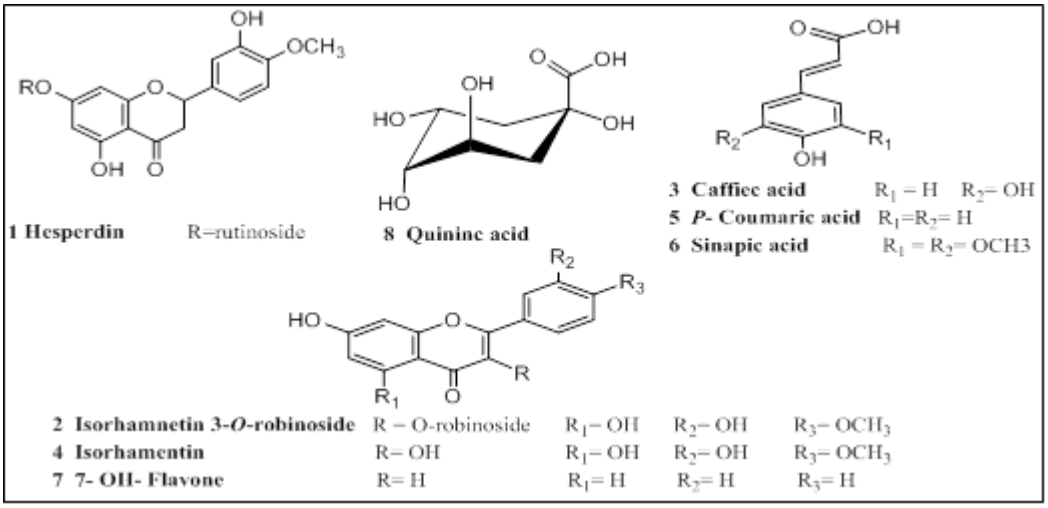

Fig (3): Chemical structure of isolated compounds from MeOH extract of *T. nudatum* aerial parts

Biological evaluation

Antitumor activity: (Viability assay)

The *in vitro* cytotoxicity activity of chloroform and methanol extracts of *T. nudatum* aerial parts investigated on three carcinoma cells HepG-2 (human hepatocellular cancer cell, HCT-116 (human colon Carcinoma) and PC-3 cells (human prostate cancer cell) recorded in the table (2). The outlined results showed that the chloroform extract exhibited potent activity against HepG-2 carcinoma cell with IC_{50} 20.7 µg/ml and moderate activity against HCT-116 and PC-3 carcinoma cells with IC_{50} 47.9 and 45.5 µg/ml respectively. Cancer is a class of diseases marked by the uncontrolled growth and spread of abnormal cells, which can lead to death if left untreated. When DNA becomes damaged in a normal cell, the cell either cures the damage or dies. The damaged DNA in cancer cells is not repaired, but in cancer cases, the cell does not die. This cell continues to produce new cells that the body doesn't require. The damaged DNA in these new cells will be similar to that of the first infected cell [11]. Apoptosis is characterized by various distinct morphological changes in the cell's structure, as well as a number of enzyme-dependent biochemical processes. The outcome is the removal of cells from the body with minimum destruction to surrounding tissues, and it is the mechanism that facilitates the work of many chemotherapeutic drugs. Apoptosis failure and the accumulation of damaged cells in the body can lead to malignant transformation and many types of cancer [23,24]. In order to find new anticancer compounds with fewer side effects or lower resistance to existing anticancer therapeutics**.** Our results recorded in the table (2) revealed that the chloroform extract has cytotoxic activity which might be helpful in preventing the cancer's progress, especially when it is compared to the activity of the methanol extract which showed no activity against all tested cells with IC_{50} 110 , 214 and 232 µg/ml for HepG-2, HCT-116 and PC-3, respectively. According to the National Cancer Institute (NCI), crude extracts from natural sources are divided into three types based on their cytotoxicity: inactive (IC_{50} >100 µg/ml), moderately active (IC_{50} 20- 100 µg/ml) and active (IC_{50} < 20 µg/ml) [25]. The results of chloroform extract showed a notable increase in inhibition % with decreasing in viability % of all the tested cells by increasing in the chloroform extract concentration. More than 60% of the all cells were inhibited at concentration < 62.5 µg of chloroform extract. By increasing the concentration of the chloroform extract to 250 µg, around 93.27, 88.72 and 85.10 % of the HepG-2, HCT-116 and PC-3 cells were inhibited respectively. Our results showed that the chloroform extract of *T. nudatum* activated the apoptosis mechanism in tumor lines. Interestingly, the chloroform extract induced the same level of cells with respect to the anti-cancer compounds, suggesting that the extract might contain a more potent compound or a mixture of compounds working in synergy as terpenoids, sesquiterpenoid, monoterpenoids, fatty acids [26,27]. poptosis of cancer cells by the activation of caspase enzymes [28]. The presence of sufficient amount of terpenoids (carvacerol, citronellol, euganol, geraniol, eduesmol, germacrene D, caryphllene and ledol) in the chloroform extract

may be linked to its antitumor activity where terpenoids have been shown to suppress the growth of numbers of cancer cells without causing any toxicity in normal cells [29,30].

Table (2): Antitumor activity of chloroform and methanol extracts of *T. nudatum* aerial parts

Conc µg/ml	Viability % of chloroform extract			Viability % of methanol extract		
	HepG-2	HCT-116	PC-3	HepG-2	HCT-116	PC-3
500	1.85 ± 0.83	4.73 ± 0.51	6.82 ± 0.36	16.21 ± 0.93	18.94 ± 1.72	24.69 ± 1.35
250	6.73± 0.65	11.28 ± 0.67	14.90 ± 0.64	28.57 ± 1.79	39.72 ± 2.64	45.34 ± 2.92
125	18.31± 0.67	23.19 ± 1.73	26.73 ± 1.51	43.28 ± 2.46	75.14 ± 3.98	77.62 ± 3.76
62.5	29.47± 1.31	38.64 ± 2.38	38.91 ± 1.75	70.39 ± 3.17	89.53 ± 1.71	92.48 ± 1.24
31.25	47.06± 3.45	62.91 ± 2.95	59.24 ± 2.38	88.04 ± 2.42	97.06 ± 0.68	99.24 ± 0.21
15.6	65.32± 2.96	76.89 ± 2.37	75.86 ± 1.76	98.15 ± 0.79	100 ± 0	100 ± 0
7.8	79.60± 1.84	85.02 ± 1.84	88.94 ± 1.38	100 ± 0	100 ± 0	100 ± 0
3.9	92.37± 1.29	94.13 ± 0.95	97.53 ± 0.96	100 ± 0	100 ± 0	100 ± 0
2	98.42± 0.64	99.74 ± 0.18	100 ± 0	100 ± 0	100 ± 0	100 ± 0
1	100± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
0	100± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
IC_{50}	**20.7 ± 2.5**	**47.9 ± 2.7**	**45.5 ± 1.4**	**110 ± 3.4**	**214 ± 5.2**	**232 ± 4.9**

Antidiabetic activity:

The *in vitro* antidiabetic activity of *T. nudatum* chloroform and methanol extracts performed to evaluate the inhibition effect of the two plant extracts on two digestive enzyme (*α*-amylase and *α*-glucosidase). The obtained results recorded in the table (3) showed that, chloroform extract has inhibition % of *α*–glucosidase better than *α*–amylase enzyme when compared with acarbose reference drug. While, the methanol extract showed no antidiabetic activity. The most common treatment for diabetes is to suppress carbohydrate digesting enzymes such *α*-amylase and *α*-glucosidase, which reduces glucose synthesis and absorption in the intestine [31]. The intestinal enzymes such as *α*-amylase and *α*-glucosidase have been discovered to play a critical role in carbohydrate digestion and glucose absorption. The suppression of these enzymes activity will delay the breakdown of starch, polysaccharides and oligosaccharides to monosaccharides before they can be absorbed, resulting in a reduction in glucose absorption, therefore, decreasing the blood glucose level [32]. The results of this study depicted in the table (3) illustrated that the chloroform extract of *T. nudatum* showed inhibition of *α*-amylase and *α*-glucosidase activity with IC_{50} 75.3 and 55.3 µg/ml**,** respectively and no inhibition activity of methanol extract against the two enzymes *α*-amylase and *α*-glucosidase with IC_{50} 470.9 and 818.2 µg/ml, respectively with comparison to standard acarbose reference drug which exhibited IC_{50} 34.71 and 30.57 µg/ml. As per the results obtained from this study, it showed that the chloroform extract exhibited an improvement of considerably inhibition activity of α-glucosidase with IC_{50} 55.3 µg/ml more than α-amylase with IC_{50} 75.3 µg/ml. According to the results obtained it showed that there was a dose-dependent increase in percentage inhibitory activity against α-glucosidase and α-amylase enzymes. The percentage inhibition value correspondingly increases with an increase in the dose concentration as shown in the respective table (3). The highest overall inhibitory effect of both *α*-amylase and *α*-glucosidase as an average of about 73.86 % and 80.19 % inhibition. Therefore, the chloroform extract of *T. nudatum* efficiently inhibits both *α*-amylase and α-glucosidase. Where, it was found that the chloroform extract responded towards α-glucosidase than that of *α*-amylase. The relative inhibition % of the two enzymes of chloroform extract may be related to the presence of phytochemical components such as terpenes [33,34], fatty acids and sterols, as many studies have been proven that phytoterpenes, phytosterols and unsaturated fatty acids have a significant activity in decreasing blood sugar level and improving the chemical and biological properties of the body [35-36].

Table (3): Antidiabetic activity of chloroform and methanol extracts of *T. nudatum* aerial parts

Conc	Mean of α – amylase inhibitory %			Mean of α – glucosidase inhibitory %		
µg/ml	Control	Chloroform	Methanol	control	Chloroform	Methanol
1000	86.32± 0.63	73.86 ± 0.63	60.49± 0.96	90.10 ±0.58	80.19 ±2.1	54.96 ±1.4
500	80.14± 0.58	68.42 ± 1.5	52.42± 1.3	86.34 ±1.2	70.85 ±0.58	41.32 ± 2.1
250	69.37 ± 1.2	61.19 ± 2.1	31.58± 0.58	71.34 ±1.5	63.23 ±1.6	17.35 ± 1.7
125	60.17 ± 0.63	56.82 ± 0.63	20.19± 2.1	63.42 ±2.1	57.89 ±2.1	9.32 ± 2.1
62.5	59.31 ± 1.5	46.25 ± 2.1	12.47± 1.4	60.14 ±0.72	51.82 ±1.6	0± 0
31.25	48.84 ± 1.2	30.18 ± 2.2	4.52± 0.58	50.31 ±1.5	38.25 ±0.82	0± 0
15.63	40.75 ± 1.5	21.46 ± 1.5	0± 0	43.28 ±1.2	29.14 ±1.4	0± 0
7.81	37.81 ± 1.2	11.25 ± 0.58	0± 0	32.15 ±0.58	19.35±2.2	0± 0
0	0 ± 0	0 ± 0	0± 0	0 ±0	0 ±0	0± 0
IC_{50}	**34.71 ± 1.3**	**75.3 ± 2.1**	**470.9 ± 082**	**30.57± 0.96**	**55.3 ± 1.3**	**818.2 ± 1.9**

Anti-inflammatory activity:

The *T. nudatum* chloroform and methanol extracts were screened for pharmacological anti-inflammatory activity by evaluating the efficacy of the two extracts as an anti-inflammatory promising drug by detection of inhibition % of cyclooxygenase enzyme and stabilizing the lysosomal membrane %. The presented data in the table (4) stated that, the methanol extract exhibited anti-inflammatory activity by stabilizing the lysosomal membrane % rather than the inhibition of cyclooxygenase % as compared by indomethacin and ibuprofen as standard drug, respectively. Pain is a symptom of many diseases requiring treatment with analgesics, it is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It can be also elicited by inflammation. Progress has been made in elucidating the role of various endogenous substances such as prostaglandins and peptides in the inflammatory process [37]. More disorders are caused by the lysosomal enzyme released during the inflammation process. These enzymes' extracellular activity is thought to be linked to acute or chronic inflammation. The Non-steroidal medicines work by either blocking lysosomal enzymes or stabilizing the lysosomal membrane [38,39]. The vitality of cells is generally recognized to be dependent on the integrity of their membranes [40]. When RBCs are exposed to harmful chemicals, such as a hypotonic medium, the membrane is dissolved, followed by hemolysis and hemoglobin oxidation [41]. The Anti-inflammatory drugs work by inhibiting the cyclooxygenase enzyme, which is responsible for converting arachidonic acid to prostaglandins (PG) and then prostaglandin G2 (PGG2) to PGH2, as well as peroxidation, which is linked to the formation of long channels in membranes. The release of chemical mediators causes the channel to open, and arachidonic acid is released from the membrane and converted to prostaglandin, which is considered as a starting point for a general inflammatory response [42]. The outlined data recorded in the table (4) illustrated that the chloroform extract showed no anti-inflammatory activity for the (COX-II enzymes and membrane stabilization) while, the methanol extract showed an eminent efficacy as an anti-inflammatory agent on the stability of the lysosomal membrane % with IC_{50} 34.9 µg/ml better of its activity inhibition % of COX-II enzyme with IC_{50} 46.9 µg/ml. The methanol extract protects the human erythrocyte membrane from lysis caused by hypotonic solution at concentrations ranging from 7.81 to 125 µg/ml. The membrane stabilization % increased with the increase of the MeOH extract concentration as shown in the table (4) where, at the concentration of 125 µg/ml, the extract produced 80.25% inhibition of RBC hemolysis as compared with 96.35% produced by indomethacin standard drug. Since HRBC membranes are similar to lysosomal membrane components, the prevention of hypotonicity-induced HRBC membrane lysis was taken as a measure of the anti-inflammatory activity of drugs [43]. So, we can say that the *T. nudatum* MeOH extract can significantly and dose-dependently inhibits RBC hemolysis. In the same manner, table (4) illustrates that the MeOH extract revealed inhibitory % of COX-II enzyme about 75.19 % for high concentration 125 µg/ml as compared with 100 % produced by ibuprofen standard drug. The anti-inflammatory activity of MeOH extract could be attributed to their phenolic, flavonoid compounds where, these compounds are known to inhibit some molecular targets of pro-inflammatory mediators in inflammatory responses [44]. The phenolic and flavonoids components found in the MeOH extract affect arachidonic acid metabolism in different ways. Some flavonoids can inhibit cyclooxygenase or lipoxygenase enzymes, whereas others can act on both [45]. Especially, hesperidin, Isorhamentin, Isorhamentin-3-O-

robinoside presence in methanol extract plays an important role in significant inhibition of prostaglandin formation and was found to be a strong inhibitor of COX-II enzymes [46,47].

Table (4): Anti-inflammatory activity of chloroform and methanol extracts of *T. nudatum* aerial parts

Conc µg/ml	Membrane stabilization %			Cyclooxygenase (COX2) inhibitory %		
	Indomethacin	Chloroform	Methanol	Ibuprofen	Chloroform	Methanol
125	96.35 ± 0.63	26.25 ±1.7	80.25 ± 0.96	100	19.35 ±1.87	75.19 ± 1.3
62.5	85.35 ±1.7	10.82 ±1.52	63.25 ± 1.3	94.31 ± 0.63	14.25 ± 0.58	61.42 ± 0.58
31.25	68.34 ±2.1	6.34 ± 0.63	48.24 ± 2.91	79.86 ± 1.2	4.62 ±0.85	38.28 ± 2.1
15.63	52.35 ±0.58	0	26.02 ± 0.86	61.21 ±0.58	0	17.36 ± 1.31
7.81	38.35 ±1.5	0	11.27 ± 0.84	31.12 ±1.2	0	10.58 ± 2.1
3.9	26.38 ±1.3	0	6.32 ± 1.4	19.35 ±0.98	0	5.27 ± 1.6
1.95	11.31 ±0.72	0	0	7.82 ±1.4	0	0
0.98	5.24 ±1.3	0	0	0	0	0
0	0	0	0	0	0	0
IC_{50}	**14.3 ± 0.74**	**>125**	**34.9 ± 1.8**	**12.7 ± 1.1**	**>125**	**46.9 ± 0.96**

Conclusion

The chemical studies performed on the *T. nudatum* extracts revealed that the plant chloroform extract was rich with terpenoids, saturated and unsaturated fatty acids components which were identified for the first time from this plant by GC-Ms analysis. Also, the contents of the methanol extract were isolated and identified by different chromatographic methods which led to the isolation of eight known compounds for the first time from this plant. The biological assessment of chloroform and methanol extracts showed the potent antitumor activity of chloroform extract toward HepG-2 and moderate activity against HCT-116 and PC-3 carcinoma cells. Also, it showed moderate antidiabetic activity with an acceptable inhibition % for both α-glucosidase and α-amylase enzymes which is related to high terpenoid contents existing in the extract that characterized by antitumor and antidiabetic efficiency. On the other side, the methanol extract of *T. nudatum* demonstrated a very good anti-inflammatory activity by increasing the lysosomal membrane stabilization and the inhibition of cyclooxygenase enzyme% which may be due to its content from phenolic and flavonoid compounds which had been reported as a very good anti-inflammatory agent. These findings alone were not enough to declare the beneficial effects of this plant species as an antidiabetic, anti-inflammatory, anti-tumor agent. It is extremely essential to also study the ability of this plant extracts to study its effects in an *in vivo* model.

Conflict of interest statement

The authors declare no conflict of interest.

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