Isolation of phytoconstituents and evaluation of anticancer and Antioxidant potential of *Launaea mucronata* (Forssk.) Muschl. subsp

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Abstract: Traditional Medicine is widely used in clinical research due to its low toxicity, low number of side effects, and low cost. Many components of common fruits and vegetables play well-documented roles as chemo preventive or chemotherapeutic agents that suppress tumor genesis. The present study was aimed to identify the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free-radical scavenging constituents from methanol extract of *Launaea mucronata* using bioassay-guided fractionation. The methanolic extract revealed a strong antioxidant activity, the IC_{50} values (the concentration with scavenging activity of 50%) was 25.94 mg/ml. The fractionation of the active methanol extract by silica gel column chromatography revealed compounds with strong antioxidant activity. The isolated bioactive compound was determined as, apigenin, quercetin, rutin, isorhamnetin-3-O-β-glucoside, quercetin-3-O-β-glucoside, apigenin-7-O- β-diglucoside, kaempferol-3-o-rutinoside), by comparing spectral data (UV, IR, ^{1}H NMR, ^{13}C NMR, and MS) with literature reports. The results obtained confirmed that methanol extract of *Launaea mucronata* or its derived phytocompounds can be used potentially as a bioactive source of natural antioxidants by contributing beneficial health effects. *Launaea mucronata* was *in vitro* investigated for cytotoxic activity against HCT116, HepG2, Hella and MCF-7 cell lines, doxorubicin was used as the reference drug. The results show IC_{50} 19, 19.60, 12.60 and 12.70 µg/ml respectively.

Keyword: Anticancer, antioxidant, *Launaea mucronata.*

INTRODUCTION

The natural defense systems of plants growing under desert stress conditions depend on certain defensive of secondary metabolites. These compounds evolve largely to deter pathogens and herbivores, like insects and mammals, whereas their levels in the plant's tissues are believed to be both environmentally induced and genetically controlled. Plants growing on low nutrient soils under drastic desert conditions, like high temperatures, intense solar radiation and water deficiency, are often more dependent on evolving chemical defenses than their counterparts growing under milder conditions. Such plants are under constant attack by reactive oxygen species and have evolved efficient antioxidant defense systems, including antioxidative enzymes or nonenzymatic antioxidants. In the desert plants, there are high concentrations of defensive metabolites, which have antioxidant, antifungal, antibacterial, antiviral and antimutagenic capabilities to the plant. An extraordinary nonenzymatic antioxidative capacity might be a characteristic of plants, which live in stressful habitat (Wang *et al*. 2007). ROS have been shown to be involved in the pathogenesis of infections, in cardiovascular and neurodegenerative diseases and the early stages of carcinogenesis (Harman, 1994, Cox and Cohen, 1996, Finkel, 2000).

Polyphenol compounds, like, phenolic acids, flavonoids, and tannins have antioxidant activity assumed to function as terminators of free radical chains or as chelators of redox-active metal ions which is capable of catalyzing**.** lipid per oxidation (Schroeter, 2002).

Launaea is a relatively small genus consists of about 40 plant species growing in dry and sandy habitats. They belong to family Asteraceae, (Ali and Qaiser 2002). Pervious work on some species of this genus proved they have antitumor, insecticide and cytotoxic activities (Rashid, *et al* 2000). The coumarin compounds isolated from *Launaea resedifolia* have antimicrobial and the neuro pharmacological properties (Ashraf and Nabil 2006, Abdu Raazag, *et al* 2007).

The main constituents compounds isolated the genus are flavonoids, coumarins [Salehh *et al* 1981, Gheraf, *et al* 2006, Giner, 1992) and trierpene compounds (Abd El-Fattah, 1990). The crude extract of *L. mucronata* had various cytotoxic activity against HCT116, HepG2 and MCF-7. New coumarin-6-isobutyl isolated from *L. Mucronata* showed high significant cytotoxic activity against different cell lines, the other new compound, coumarin-6-isobutyl-7-methyl, had high cytotoxity against HCT116 (El-Sharkawy and Mahmoud, 2015).

Lupeol, lupeol acetate and β-sitosterol were isolated from petroleum ether extract of aerial parts of *Launaea mucronata* grown in Egypt (Abdel-Slam *et al*, 1982). Additionally, the roots of the same sample yielded the sesquiterpene lactone, guaianolide lactucin, dihydroderivative 11β-13-dihydrolactucin, and 8-acetyl-11β, 13-dihydrolactucin (Sarg, 1982).

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Present study was aimed to isolate active compounds from plant, which distribute and grow under dry desert condition and study the antioxidant and anticancer property of methanol extract of *Launaea mucronata* through bioassay-guided fractionation using in vitro DPPH assay method.

MATERIAL AND METHODS

Plant material

Flowering aerial parts of *Launaea mucronata* were collected from the wild population growing in El-Qantarasharq-North Sinai, were collected in spring 2013, the identity of the plants have been kindly verified in, Faculty of Science, Northern Border University. Voucher specimens [SgLm12], were deposited in the herbarium of the faculty of Science, Northern Border University, girl department.

Isolation and identification of flavonoid compounds

Extraction

Aerial parts of plants (1kg) were air-dried at room temperature in the shade and grinded to powder. The powders were subjected to *extraction* by percolation in 90% methanol and filtered-off, the marc lifted was extracted by the same way (this process repeated four times). The combined methanol extracts were concentrated under reduced pressure at a temperature not exceeding 40^{o}C till dryness.

The dried extract was dissolved in small amount of water and extracted successively using, chloroform, ethyl acetate and n-butanol by separating funnel, each extract was dried over anhydrous sodium sulfate and concentrated again as before, to give 3.5g, 5.2g and 8.5g, chloroform, ethyl acetate and n-butanol extracts respectively.

Isolation and purification

By testing all fractions ethyl acetate was containing the major and promising spots revealed to flavonoids compound, ethyl acetate fraction (8.5g) was subjected to silica gel, column chromatography, eluted with 100% chloroform and the polarity increased gradually with ethyl acetate and methanol. Fractions (200 ml each) were collected; collective fractions were obtained according to TLC manner using the system; ethyl acetate: methanol: water (30: 5: 4). Six collective fractions (L1-L6), were obtained containing seven major flavonoid compounds. The sub-fractions were subjected to thin layer chromatography (TLC) using system c, The final purification of some compound was carried out by, re-chromatography on a Sephadex LH-20 column with methanol (MeOH) as the eluting solvent to obtain compounds 1 (15 mg), 2 (25 mg), 3 (16 mg) and 4 (20 mg) in pure form. L5 fraction was subjected to silica-gel chromatography using eluted with 100% chloroform and the polarity increased gradually to 100% methanol. Pure compounds were rechromatographed on a Sephadex LH-20 column to obtain compound 5 (18 mg), 6 (15 mg), 7 (12 mg) in pure form. The pure compounds were crystallized from methanol. Identification and structure elucidation of the purified flavonoid compounds were done by, R_f values in paper chromatography, spectral data H^1NMR (Varian 600 MHz) and C^{13}NMR. The sugar moiety was identified by partial and complete acid hydrolysis using PC with authentic samples.

General experimental conditions

Thin layer chromatography (TLC) (Silica gel G-60 F254 Merck). Column chromatography (Silica gel G-60, 70-230 mesh). Paper chromatography (Whatman No. 3). Nuclear Magnetic Resonance (NMR) spectra were measured at 600.17 and 150.91 MHz for ^{1}H- and ^{13}C-NMR respectively.

Solvent systems; a: Chloroform -methanol (9:1), b: ethyl acetate-methanol - water (30:5:4), c: ethyl acetate - methanol - acetic acid - water (65:15:10:10) and butanol - acetic acid - water (4:1:5 upper layer) were used. Chromatograms were achieved under UV (254 and 365 nm) before and after exposure to ammonia vapor or by spraying with aluminum chloride.

Compound 1: Apigenin

(15 mg), faint yellow crystals, R_f = 0.9 (system a) and 0.058 (system b), mp 348-349°C. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.93 (1H, d, J = 8 Hz, H2$^{/}$ & H6$^{/}$), 6.92 (2H, d, J=8 Hz, H3$^{/}$ and H5$^{/}$), 6.77 (1H, d, J=2.5 Hz, H8), 6.47 (1H, S, H3), 6.21 (1H, d, J=2.5 Hz, H6). ^{13}C NMR (DMSO): δ (ppm): 182.21 (C-4); 164.19 (C-2); 163.31 (C-7); 161.95 (C-5); 161.79 (C-4$^{/}$); 157.86 (C-9); 128.98 (C-2$^{/}$ & C-6$^{/}$); 121.63 (C-1$^{/}$); 116.51 (C-3$^{/}$ & C-5$^{/}$); 105.83 (C-10); 103.30 (C-3); 99.89 (C-6); 94.55 (C-8). Molecular formula $C_{15}H_{10}O_5$

Compound 2 : Quercetin

(25 mg) slightly yellow powder; MP. 316 °C; 1H NMR δ (ppm) =6.12 (1H, *d, J*=2.0 Hz, H-6), 6.29 (1H, *d, J*=2.0 Hz, H-8), 6.79 (1H,*d, J*=8.3 Hz, H-5$^{/}$), 7.64 (1H, *dd*, *J*= 8.3; 2.1 Hz, H-6$^{/}$), 7.77 (1, *d*, *J*=2.1 Hz, H-2$^{/}$). ^{13}C NMR δ (ppm) = 94.4 (CH, C-8), 99.1 (CH, C-6), 103.7 (C, C-10), 116.1 (CH, C-2$^{/}$, C-5$^{/}$), 120.8 (CH, C-6$^{/}$), 123.3 (C, C-1$^{/}$), 137.2 (C, C-3), 145.3 (C, C-3$^{/}$), 148.2 (C, C-2), 150.3 (C, C-4$^{/}$), 158.4 (C, C-9), 162.6 (C, C-5), 165.7 (C, C-7), 177.5 (C, C-4); Molecular formula **$C_{15}H_{10}O_7$**.

Compound 3: Rutin

(16 mg) was obtained as dark brown particles with R_f = 0.24 (system a) and 0.57 (system b). ^{1}H NMR: δ (ppm) =6.19(1H, d, J=2, C6-H), 6.38 (1H, d, J=2, C8-H), 7.52 (1H, d, J=2.1,C2-H), 6.76 (1H, d J=9,C5$^{/}$-H),7.55 (1H, dd, J=9,2.1, C6$^{/}$-H),9.71 (1H, s, C4$^{/}$-OH), 9.21 (1H, s, C3$^{/}$-OH), 12.62 (1H, s, C5-OH), 10.86 (1H, s, C7-OH),

5.35 (1H, d, J=7.4, H1-G),5.12 (1H, d, J=1.9, H1-R), 1.00 (3H, d, J=6.1,CH3-R); ^{13}C NMR (chemical shift δ in ppm) 157.3 (C-2),134.1 (C-3), 178.2 (C-4), 157.5 (C-5), 99.5 (C-6), 164.9 (C-7), 94.5 (C-8), 162.1 (C-9), 104.8 (C-10), 122.5 (C-1′), 116.1(C-2′), 145.3 (C-3′), 148.3 (C-4′), 117.1 (C-5′), 122.0 (C-6′),101.6 (C1-G), 74.9 (C2-G), 77.3 (C3-G), 72.7 (C4-G), 76.7 (C5-G), 67.9 (C6-G), 102.2 (C1-R), 70.8 (C2-R), 71.0 (C3-R), 71.3 (C4-R), 69.2 (C5-R), 18.4 (C6-R) [R and G represent signals from rhamnose and glucose moieties, respectively]. Molecular formula $C_{27}H_{30}O_{16}$.

Compound 4: Isorhamentin-3-O-B-D-glucpuranoside

(20 mg), yellow crystals, R_f=0. 59,^{1}H NMR (chemical shift δ in ppm): δ 7.8(1H, d, J=2.5Hz, H-2`), 7.5 (1H, dd, J= 2.5, 8.5Hz, H-6`), 6.8 (1H, d, J= 8.5Hz, H-5'), 6.3(1H d, J 2Hz, H-6), 6.1(1H d, J 2Hz, H-8) 3.7(3H s,. OCH3), Sugar: δ 5.5 (1H, d, J=7.4Hz, H-1" glucose), remaining sugar protons are evident at 3-4. ^{13}C NMR (DMSO) (ppm): compound: 161.77 (C-2), 133.08 (C-3), 178 (C -4), 115.86 (C-5), 99.34 (C-6), 164 (C-7); 94.34 (C-8), 122.61 (C-6'), 114.30 (C2', 5'), 101.4 (C-1"), 72.8 (C-2"'), 77.5 (C-3"'), 69.8 (C-4"'), 76.2 (C-5"'), 61.0 (C-6"'),59 (O-CH3). Molecular formula, $C_{22}H_{22}O_{12.}$, (Chen, 2005).

Compound 5 kaempferol-3-O-rutinoside

(18 mg), Yellow amorphous powder, UV λ(max) nm: 225, 270, 350 (MeOH). ^{1}H NMRδ 7.98 (2H, d, *J*=8.7 Hz, H-2′, 6′), 6.88 (2H, d, *J* = 8.7 Hz, H-3′, 5′), 6.40 (1H, br.s, H-8), 6.20 (1H, br.s, H-6), 5.30 (1H, d, *J*=6.9 Hz, H-1″), 4.39 (1H, br.s, H-1‴), 3.0 ~ 4.0 (16H, rut), 1.10 (3H, d, *J*= 6.4 Hz, -CH3). ^{13}C NMRδ: 156.4 (C-2), 133.1 (C-3), 177.2 (C-4), 161.1 (C-5), 98.7 (C-6), 164.0 (C-7), 93.7 (C-8), 156.7 (C-9), 103.7(C-10), 120.7 (C-1′), 130.7 (C-2′, C-6′), 115.0 (C-3′, C-5′), 159.8 (C-4′), 101.3 (C-1″), 74.1 (C-2″), 76.3(C-3″), 69.8 (C-4″), 75.6 (C-5″), 66.8 (C-6″), 100.7 (C-1‴), 70.2 (C-2‴), 70.5 (C-3‴), 71.7 (C-4‴), 68.1(C-5‴), 17.6 (C-6‴). All data were identical with that of kaempferol-3-O-rutinoside). Molecular formula, $C_{27}H_{30}O_{15}$ (Ahmed *et al.,* 2008)

Compound 6: Apigenin-7-O-diglucoside

(15 mg) was obtained as dark brown particles with R_f = 0.22 (system a) and 0.56 (system b). UV λ_{max} MeOH: 256, 267, 389; (NaOAc) 268, 395; (NaOAc/H_3BO_3); 268, 336; ($AlCl_3$) 277, 300, 345, 384; ($AlCl_3$/HCl) 277, 299, 342, 382. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.9 (2H, d, *J* = 8.8, H-2′, 6′), 6.8 (2H, d, *J* = 8.8, H- 3', 5'), 6.8 (1H, s, H-3), 6.79 (1H, d, *J*=2.0, H-8), 6.4 (1H, d, *J*=2.0, H-6), 5.0, 5.2 (1H, d, *J* =8, 2H-1″), 3-4 (remaining sugar proton, m). ^{13}C NMR (DMSO) (ppm): 164.3 (C-2), 103.5 (C-3), 182.4 (C-4), 160.9 (C-5), 100.4 (C-6), 162.6 (C-7), 94.1 (C-8), 160(C-5″), 61.0 (C-6″), 101.4 (C-1‴), 72.8 (C-2‴), 77.5 (C-3‴), 69.8 (C-4‴), 76.2 (C-5‴), 61.0 (C-6‴).

Compound 7: Quercetin-3-O-β- Dglucopyranoside

Yellow amorphous powder, mp 231-232ºC.^{1}HNMR δ 12.64 (1H, s, 5-OH), 7.59 (1H, d, *J*=2.5 Hz, H-2′), 7.57 (1H, dd, *J*=8.5,2.5 Hz, H-6′), 6.84 (1H, d, *J*=8.7 Hz, H-5′), 6.40 (1H,d, *J*=2.5 Hz, H-8), 6.20 (1H, d, *J*=2.5 Hz, H-6), 5.47(1H, d, *J*=6.9 Hz, H-1″), 5.4 ~ 3.0 (10H, m, glc). All data were identical with that of quercetin-3-*O-β*-Dglucopyranoside, Molecular formula, $C_{21}H_{20}O_{12}$ (Belboukhari and Cheriti 2006, Chen, 2005).

Pharmacological studies

DPPH radical scavenging activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by (Braca, 2001) 0.1ml different extracts in different concentrations (100mg/ml to 8mg/ml) were added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517nm was determined after 30 min, and the percentage inhibition activity was calculated from this Equation, [(A0–A1)/A0] x100, where A0 is the absorbance of the control, and A1 is the observance of the extract/ standard (Ascorbic acid).

Cytotoxic assay

Cytotoxic effect on human cell line (HePG 2 – MCF 7 – HCT 116- A549)

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan (Mosmann, 1983).

Procedure: All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium for HePG2- MCF7 and HCT116 – DMEM for A549. The media are supplemented with 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B), 1% L-glutamine and 10% fetal bovine serum and kept at 37 ºC under 5% CO_2.

Cells were batch cultured for 10 days, then seeded at concentration of $10x10^3$ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ºC for 24 h under 5% CO2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (50-25-12.5 and 5 ug/ml). After 48 h of under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200 µL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37ºC. The

absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference incubation, medium was aspirated, 40ul MTT salt (2.5µg/ml) were added to each well and incubated for further four hours at 37ºC wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula:
((Reading of extract / Reading of negative control)-1) x 100

STATISTICAL ANALYSIS

All values were expressed as the mean of percentage of inhibition cells of the four replicates for each treatment. Data were subjected to SPSS (ver.8.0). $P<0.05$ was regarded as significant. Each value represents the mean of percentage of inhibition cells of three replicates ± SEM (Standard error of mean).

RESULTS

The methanol extract of *Launaea mucronata* was initially screened by spectrophotometric DPPH assay and the results are shown in fig. 2. The DPPH assay revealed that the methanol extract had significant scavenging effects with increasing concentrations in the range of 10-50µg/mL. The scavenging activity of extract (50 %) was less than that of ascorbic acid (96%). and the IC_{50} values (the concentration with scavenging activity of 50%) were 25.94 94 mg/ml □ (fig. 2). Hence, the ME (methanol extract), was subjected to repeated bioassay-guided fractionation on silica gel column chromatography using petroleum ether: EtOAc gradient elution system. The further purification of active fractions obtained from silica gel column chromatography finally yielded a bioactive flavonoid compounds from the Hexane-ethyl acetate (85:□15 v/v) mixture.

Seven flavonoids were identified, and their structures were elucidated**,** asapigenin (1), quercetin (2), rutin(3), isorhamintine-3-glucoside (4), kaempferol-*3- O*-rutinoside (5), apigenin -*7-O*-β-Di–glucopyranoside (6), and quercetin-3-*O*-β-D-glucopyranoside (7), on the basis of chemical and physicochemical evidence. The compounds were identified by UV, ^{1}H, $^{13}CNMR$ and then comparison of their spectroscopic data with previously reported values [19.20].

All the compounds shown in fig. 1, were isolated for the first time from this plant, wherever, rutin (4), quercetin-3-*O*-β-D-glucopyranoside (5), isorhamintine-3-glucoside (6), apigenin -*7-O*-β-Di-glucopyranoside were isolated more from genus and family. (Mansour *et al* 1983)

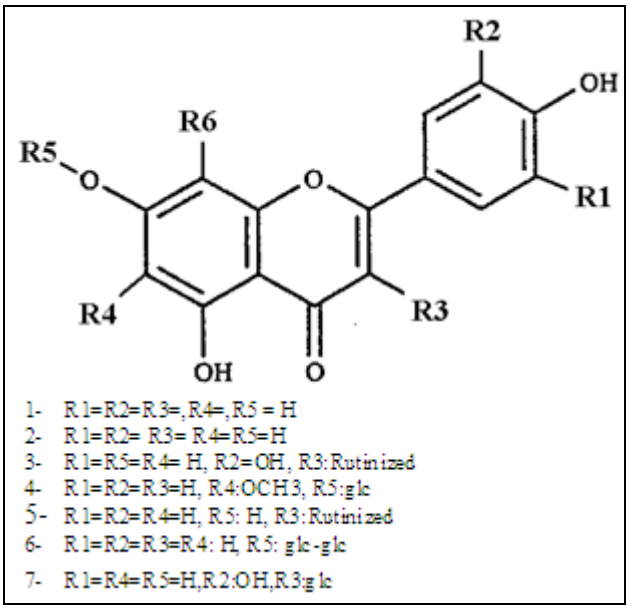

Fig. 1: Strucure of isolated compounds

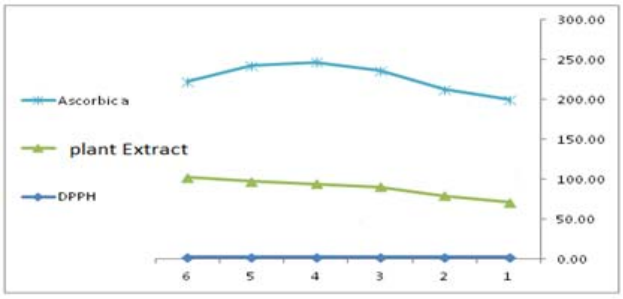

Fig. 2: Antioxidant activity of extract and ascorbic acid (0.1M concentration) in vitro, using DPPH radical scavenging activity method.

Antioxidant activity

To study the antioxidant activity of methanol extract of *Launaea,* its scavenging activities was tested on DPPH radicals. The results are presented in, fig. 2 as a percentage of DPPH radical scavenging activity.

DPPH test is a direct and reliable method for determining radical scavenging action. The DPPH radical contains an odd electron, which is responsible for absorbance at 515-517 nm and also for a visible deep purple color. When DPPH gains an electron donated by an antioxidant compound, the DPPH is decolorized, that can be quantitatively measured from the changes in absorbance. The ratio of antioxidant/DPPH required to decrease the concentration of DPPH to 50% of its initial value, denoted as EC_{50} (Efficient Concentration), is an indicator of antiradical activity, i.e. the lower the EC50, the more potent the scavenging activity. The EC_{50} values of MeOH extract was 25.94mg/ml. The scavenging activity of extract (50%) was less than that of ascorbic acid (96%).

Table 1: Cytotoxicity of methanol extract against different cultured human cell line *in vitro*

% of Inhibition ± SEM				
Human Cell line Conc. (µg/ml)	HCT116	HepG 2	MCF7	Hela
IC_{50} (µg/ml)	19	19.6	12.3	12.7
50	77.50±0.03	83.91±0.02	59.32±0.01*	79.72±0.01*
25	63.21±0.02	59.22±0.01	66.60± .06*	59.10±0.06*
12.5	35.41±0.001	37.55±0.0003	50.30±0.06*	37.74±0.01*
5	22.10±0.01	20.80±0.01*	36.81±0.06*	19.80±0.05*

Cytotoxic assay

The cytotoxic activity of methanolic extract of *Launaea* was assessed *in vitro* against, Hela, HCT116, HepG2 and MCF7 cell lines. The percentages of inhibition related to the reference drug (doxorubicin) are shown in table (1). The methanol extract at concentration 50 and 25µg/ml give very high cytotoxicity against HepG2 with ratio 83.91%, while against Hela cell line at concentration 25µg/ml gives good cytotoxic activity 66.60% and good cytotoxicity against HCT116 at 50 µg/ml, while at low concentration, methanol extract showed normal activity against all cell lines when compared with doxorubicin. The high activity of extract against different cell lines, may be related to the major component (flavonoid and phenolic compounds) in plant extract. The isolated compounds were studied before and many references show most of these flavonoid compounds have cytotoxic activity against different cell lines. *In vitro* cytotoxicity of quercetin against HCT116 cell line, show IC50 was 20.1µg/ml, while, for quercetin 3-*O*-*β*- glucoside, isorhamnetin 7-*O*-*β*-glucoside, apigenin 3-*O*-*β*-rhamnoside and kaempferol 3-*O*-*β*-glucoside, were 24.3, 22.8, 23.4 and 41.9µg/ml, respectively (Mohammed, *et al.,* 2011)

DISCUSSION

Reactive oxygen species (ROS), including free radicals such as super oxide anion radicals, hydroxyl radical species, singlet oxygen, and hydrogen peroxide, are active oxygen species that are often generated by biological oxidation reactions of exogenous factors, (Crutti, (1990;Halliwell and Gutteridge, 1990). These ROS are known to cause damage to biological molecules (Kehrer, 1993), more phenolic compounds isolated from medicinal plants can trap the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes (Lewis, 1993; Rong and Zeyuan 2004).

Seven flavonoids were identified, and their structures were elucidated as apigenin (1), quercetin (2), rutin(3), isorhamintine-3-glucoside (4), kaempferol-*3*- *O*-rutinoside (5), apigenin -*7*-*O*-β-Di–glucopyranoside (6), and quercetin-3-*O*-β-D–glucopyranoside (7), on the basis of chemical and physicochemical evidence. The compounds were identified by UV, ^{1}H, $^{13}CNMR$ and then comparison of their spectroscopic data with publish reported values (Belboukhari and Cheriti, 2006) Chen *et al*., 2005).

The methanol extract of *Launeae* shows high antioxidant activity, moreover it is rich with favonoid compounds, biogudedassy fractionation revaled the isolation of sevene flavonoid compounds, apginen, Rutin and querctine, kampferol-3-O-glucoside, which were used as a natural antioxidants. More studies on medicinal plants confirm the relation between the antioxidant activity and the presence of polyphenolics content. (Oke and Hamburger, 2002) study the antioxidants of the Nigerian plants by DPPH test and the major constitutents are polyphenolics compounds. Polyphenolics compound function as scavengers of free radicals by rapid donation of a hydrogen atom to radicals, many phenolics, such as flavonoids, have antioxidant capacities that are much stronger than those of vitamin C and E (Prior and Cao, 2000; Danuta *et al*., 2010). In these studies, the antioxidant activity of rutin and buckwheat-originated material exhibited ability to scavenge different types of free radicals. The application of cyclic voltammetry showed dependence of the antioxidant activity of rutin on the first and second oxidation potentials and was found a useful tool for the evaluation of the antioxidant capacity of buckwheat material. A 80µg/ml of isolated quercitrin and ascorbic acid exhibit 90.46% and 96.30% inhibition against DPPH respectively and IC value 50were found to be 6.167µg/ml and 4.321µg/ml for isolated compound and ascorbic acid respectively. (Sankhadip *et al*., 2013) from these reviews we can support the used of plant as antioxidant and anticancer where it is rich with bioactive phenolic compounds.

CONCLUSION

On the basis of the results obtained in the current study, it is concluded that a methanol extract of *Launaea mucronata* has high cytotoxity against HepG2 cell lines. Also, methanolic extracts of plant have a good antioxidant activities *in vitro* assays indicating that, plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigation was carried out to isolate and identify the antioxidant compounds present

in the plant extract. Methanol extract rich with flavonoid compounds, seven flavonoids compounds were isolated, and purified using sephadex LH-20 column chromatography and preparative paper chromatography. The results of this work support the use of *Launaea mucronata* in traditional medicine.

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