



CODEN [USA]: IAJPBB

ISSN: 2349-7750

**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES**<http://doi.org/10.5281/zenodo.1254385>Available online at: <http://www.iajps.com>

Research Article

**NEW SECONDARY METABOLITE AND BIOACTIVITIES OF
ASPHODELUS REFRACTUS****Mohamed F. Abdelwahab^{1,2*} and Mohamed A. Ashour^{1,2}**¹ Department of Phytochemistry and Natural Products, Faculty of Pharmacy, Northern Border University, Rafha- 91911, P.O. BOX 840, Kingdom of Saudi Arabia.² Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Egypt.**Abstract:**

An investigation of the chemical constituents of the ethanolic extract of *Asphodelus refractus* herb, led to the isolation of new 1,3,4-trihydroxy-8-methoxy-2-methyl anthraquinone- Refractin(2), together with four known Compounds 1,3,4, and 5 were identified as Chryseophanol, Luteolin, p- hydroxyphenethyl trans-ferulate and 7 hydroxy emodin, respectively. Their chemical structures have been deduced on the basis of 1D (¹H and ¹³C), 2D (HMQC, and HMBC) NMR, UV, IR and ESI/MS spectral studies. The total extract and Ethyl acetate extract were evaluated for their cytotoxic activity against human Hepatocellular carcinoma (HepG-2), Colon carcinoma (HCT-116), and Lung carcinoma (A-549) cell lines. The antioxidant activities were also evaluated using 1, 1-diphenyl-2-picrylhydrazyl free radical (DPPH), assay method. The measurement of polyphenolics by folin - ciocalteau reagent and flavonoid by NaNO₂-AlCl₃-NaOH indicated that 30 mg rutin and 52 mg gallic acid equivalents/1gram of powdered *Asphodelus refractus* herb, respectively.

Keywords: *Asphodelus*, Refractin, Antimicrobial, Antioxidant, Cytotoxicity.**Corresponding author:****Mohamed F. Abdelwahab,**

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Please cite this article in press Mohamed F. Abdelwahab and Mohamed A. Ashour., *New Secondary Metabolite and Bioactivities of Asphodelus Refractus*, Indo Am. J. P. Sci, 2018; 05(05).

INTRODUCTION:

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicine, folk medicine, food supplements, nutraceuticals, pharmaceutical intermediates and chemical precursors for synthetic drugs. Information of the chemical constituents of plants is desirable for the discovery of therapeutic agents and discovering the actual value of folkloric remedies [1].

Phytochemical studies often make use of accumulated ethnobotanical knowledge, particularly the uses of the plants in traditional medicine. The Kingdom of Saudi Arabia was characterized by many wild plants. For example 1586 plant species belonging to only 23 genera, or about 15.4% of the total plant genera [2].

The genus *Asphodelus* belongs to the family Liliaceae which comprises 187 genera and 2500 species. It is a Mediterranean genus, which includes five sections and is represented by 16 species. *Asphodelus* is a stout robust herb with roots of several spindle-shaped tubers, widely distributed over the coastal Mediterranean region. Its bulbs and roots are used to treat ectodermal parasites, jaundice, psoriasis and microbial infections. Lipids, carbohydrates, sterols, triterpenes, anthraquinones and arylcoumarins have been isolated from *Asphodelus*. Anthraquinones and pre-anthraquinones are considered important chemotaxonomic markers for plants in the family [3].

Asphodelaceae. Naphthalenes co-exist or couple with anthraquinones and pre-anthraquinones, thus indicating their biogenetic relationship. Anthraquinones are a class of natural compounds that consist of several hundred compounds that differ in the nature and positions of substituent groups. This class of compounds contains derivatives that consist of the basic structure of a 9, 10-anthraquinone moiety [4-5].

On surveying the literature, anthraquinones, flavonoids, oxepines, and sesquiterpene lactones have been reported to occur in the genus *Asphodelus* [6].

Anthraquinones are reflected important chemotaxonomic markers for plants in the family Asphodelaceae. Anthraquinones are widely applied in medicine, food and the dye industry. Anthraquinones are a class of natural compounds that consists of several hundreds of compounds that differ in the nature and positions of substituent groups. Anthraquinones can be divided into emodin and alizarin types based on two main biosynthetic pathways. The alizarin types are formed through

succinyl benzoic acid pathway and only have one of the rings unsubstituted. The emodin types are made through the polyketide pathway known as acetate-malonate pathway and have both rings substituted [7-8].

Traditionally, *Asphodelus* species used for treatment of colds and hemorrhoids, a febrifuge, anti-tumoral, diuretic, emmenagogue and rheumatic pain. The extracts of different *Asphodelus* species showed antioxidant properties, herbicidal, antibacterial and antifungal activity [9-12].

MATERIALS & METHODS:

Plant material

The aerial parts of *Asphodelus refractus* (385 g) were collected from Lena valley, Northern border, Saudi Arabia. The plant was identified by the Department of Phytochemistry and Natural products, Northern Border University, Faculty of Pharmacy, Rafha.

Preliminary Phytochemical investigation

The chemical tests were carried out on the alcoholic extract using the procedures outlined by Harborne, Trease and Evans. [13-14]

The tests were performed for Tannins (FeCl₃), Saponins (Froth), Alkaloids (Wagner's and Dragendorff's), Flavonoids (NaOH), Carbohydrates/Glycosides (Molisch, Fehling), Triterpenoids (Liebermann burchard) and Anthraquinones (modified Borntrager's).

General spectral analysis

Melting points were obtained on a Stuart SMP3 apparatus. UV spectra were determined with UV-spectrophotometer (APEL PD-303UV). IR was carried out on Perkin Elmer 78 infra-red spectrophotometer. ESIMS (positive ion acquisition mode) was carried out on an XEVO TQD triple quadrupole instrument (Waters Corporation, Milford, MA 01757, USA) mass spectrometer. Proton nuclear magnetic resonance (1H NMR) and 13C NMR spectra were obtained in dimethyl sulfoxide (DMSO)-d₆ on a Varian Gemini 400 MHz spectrometer and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (J) in Hertz. Si gel (Si gel 60, Merck), were used for open column chromatography.

Extraction and Isolation:

The air-dried powdered *Asphodelus* (385g) was exhaustively extracted with 70% methanol. The combined extracts were concentrated under vacuum at 40°C to dryness (35 g). The latter was fractionated by vacuum liquid chromatography (VLC), using n-hexane, ethyl acetate, methanol. The ethyl acetate

fraction was fractionated over Si gel column successively eluted with n-hexane and a gradient of n-hexane: ethyl acetate and finally with ethyl acetate: methanol (3:1) to afforded fractions F1 to F5 respectively.

Fraction F2 (5g.) was chromatographed using sephadex column chromatography and $\text{CHCl}_3/\text{MeOH}$, (4:6) as mobile phase to afforded three sub-fractions, F2a (0.45g), F2b(2.98 g), and F2C (1.3 g).

Subfraction F2C was further chromatographed using sephadex column chromatography and $\text{CHCl}_3/\text{MeOH}$, (3:7) as mobile phase to afforded four sub-fractions R1 to R4. R1 was purified using silica gel chromatography and n.hexane: ethyl acetate (8:2) as mobile phase to afforded compound **1**, while R4 was also purified using silica gel chromatography and n.hexane: ethyl acetate (8:2) as mobile phase to afforded compound **2**.

Fraction F3 (5.1 g) was subjected to subsequent purification on Sephadex column chromatography and $\text{CHCl}_3/\text{MeOH}$, (4:6) as mobile phase to afforded three additional compounds **3**, **4**, and **5** respectively.

Quantitative analysis of extract

Determination of total flavonoid content by $\text{NaNO}_2\text{-AlCl}_3\text{-NaOH}$ spectrophotometric method:

Firstly, 1 mL of flavonoids extracts was accurately adding to 2 mL of ultra-pure water and 0.3 mL of the NaNO_2 (5 %) solution, shaken and left to stand for 5 min. Secondly, 0.3 mL of the AlCl_3 (5 %) solution was added, shaken. Lastly after 5 mins 2 ml (1M) of NaOH was added and adjusted up to 5 ml with water and absorbance was measured at 510 nm using UV spectrophotometer (APEL PD-303UV). The total % of flavonoid content was calculated from calibration curve of rutin (5-50 μg) by plotting graph absorbance vs concentration. Total flavonoids were expressed as rutin equivalents in milligrams per gram of sample.[15-16]

Determination of total polyphenolic content by Folin-Ciocalteu spectrophotometric method:

Firstly, 1 mL of extract was accurately adding to 1 mL of ultra-pure water and 1 ml of Folin-Ciocalteu's (1/10) reagent, shaken and left to stand for 5 min. Secondly, 1 mL of the Na_2CO_3 (10 %) solution was added, shaken. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 750 nm with an UV-Vis spectrophotometer (APEL PD-303UV). The total % of phenolic content was calculated from calibration curve of Gallic acid by plotting graph absorbance vs

concentration. Total phenolic content was expressed as mg Gallic acid Equivalents. [15-16]

Biological investigations

Antimicrobial Activity

Agar well diffusion method was used to investigate Antimicrobial activity. The test sample was used in concentration of 20mg/mL and zones of inhibition were measured mm \pm standard deviation. The activity of tested samples was studied against the *Staphylococcus aureus* (AICC25923) and *Bacillus subtilis* (NCTC8236) while *klebsiella pneumonia* (AICC27853), *Escherichia coli* (AICC25922), and *Candida albicans* (AICC7596).

Antimicrobial tests were carried out by the agar well-diffusion method for tested bacteria and fungi spread on nutrient agar and malt extract agar, respectively. After the media had cooled and solidified, wells 6 mm were made in the solidified agar and loaded with 100 μL of tested sample solutions in 1 mL DMSO with concentrations of 10 mg/ml. Negative controls were prepared using DMSO employed for dissolving the tested samples while gentamycin, and amphotericin B were used as positive controls for Gram-positive bacteria, Gram-negative bacteria, and fungi, respectively. The inoculated plates were then incubated for 24 h at 37°C for bacteria and 48 h at 28°C for fungi and the diameter of any resulting zones of inhibition of growth was measured in millimeter (mm).[17]

Evaluation of antioxidant activity

The stock solutions of extracts were prepared in methanol to achieve the concentration of 1 mg/ml. In brief, 0.1 mM solution of DPPH in methanol was prepared. This solution 1 ml was added to 3 ml of different extracts in methanol at different concentration 5, 10, 15, 20, 25 $\mu\text{g}/\text{ml}$. The mixture was shaken vigorously and after 30 min of incubation in darkness at room temperature, the absorbance was recorded by using Vis spectrophotometer at 517 nm. Reference ascorbic acid was used as standard and experiment was done in triplicate.

The IC_{50} value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity.

The percent DPPH scavenging effect was calculated by using following equation: DPPH scavenging effect % inhibition = $1 - (\text{Absorbance of sample} / \text{Absorbance of control}) \times 100$.

Cytotoxicity assays

The ethanolic extracts were tested for cytotoxicity against three human tumor cell lines: Human Colon cancer cells (HCT-116), Human liver cancer hepatoma carcinoma cells (HepG-2), and Human lung carcinoma (A-549) cell lines. The cells were obtained from the American Type Culture Collection (ATCC, USA). The cells were grown on Roswell Park Memorial Institute 1640 medium (Nissui Pharm., Japan) supplemented with inactivated fetal calf serum and gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO₂ and were sub cultured 2–3 times a week. The cytotoxic activity was determined using cell viability assay method. The cell viability was calculated as the mean absorbance of control cells/mean absorbance of treated cells. Dose-response curves were prepared and the IC₅₀ value was determined. [19-20]

Acute Toxicity Study

Acute toxicity studies were performed according to OECD-423 guidelines category IV substance (acute toxic class method). Albino mice (n=3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 hrs with free access to water only. The extract was administered orally with an initial dose of 1000 mg/kg body weight. The mortality was observed for three days. If mortality was observed in 2/3 or 3/3 of animals, then the dose administered was considered as a toxic dose. However, if the mortality was observed only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher dose (OECD-423). [21]

Determination of the analgesic activity

The analgesic activity of hydroalcoholic extracts was determined by hot plate methods.

The paws of mice are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. A cut-off period of 15 sec was observed to avoid damage to the paws. The mice were randomly divided into five groups, A,B,C,D and E. Ugo Basile Analgesia hot plate consisted of a metal surface which heated electrically to 55 ± 0.1 °C and foot pedal is pressed to start a clock until licking / jumping starts.[22]

Preparation of test samples

1 gm of dried hydro-alcoholic extracts is emulsified by 2 ml tween 80 and is completed to 50 ml with distilled water to obtain 2 % w/v solutions of *extract*.

Doses of 100, 200 and 300 mg/kg through giving 0.1, 0.2 and 0.3 ml per 20gm of mice body weight respectively.

Statistical analysis

All values were expressed as mean±SD and compared with the corresponding control values. P- Values are calculated by using one – way ANOVA followed by Tukey multiple comparison tests. P <0.05 was regarded as statistically significant.[23]

RESULT AND DISCUSSION:

Phytochemical screening

The phytochemical screening of ethanolic extract revealed that contain Anthraquinones, carbohydrates, tannins and triterpenes/sterols while alkaloids and Saponins were not present.

Isolation of the compounds

Compounds **1**, **3**, **4**, and **5** were identified as Chryseophanol, Luteolin, of *p*- hydroxyphenethyl trans-ferulate, and 7 hydroxy emodin respectively through comparison of spectral data (MS, ¹HNMR, ¹³CNMR, HMQC, and HMBC, with those previously reported in literature's; while structure of compound **2** was confirmed (MS, ¹HNMR, ¹³CNMR, HMQC, and HMBC) To the best of our knowledge this is the first report for isolation of this compound in nature.

Compound 1 was obtained as a yellowish red amorphous powder and gave on TLC [*R_f* = 0.75, using pre-coated silica gel plate and n.hexane: ethyl acetate (8:2)] deep red spot after fuming with NH₃ vapor indicating an anthraquinone derivative. The Compound 2 shows a pseudo-molecular ion peak at 255 m/e [M+H]⁺ based on the positive ESI/MS analysis, which is consistent with the molecular formula C₁₅H₁₀O₄. The ¹³C NMR, DEPT, and HMQC of Compound **1** displayed 15 carbon signals, including one methyl group at δ 22.2 (on C-3), 5 methines at δ 124.33 (C-2), 121.32 (C-4), 119.9 (C-5), 136.92 (C-6) and 124.52 (C-7) and 7 quaternary carbons at δ 162.8 (C-1), 149.3 (C-3), 162.5 (C-8), 113.8 (C-1a), 133.7 (C-4a), 133.3 (C-5a), and 115.9 (C-8a) as well as two carbonyl carbons at δ 192.5 (C-9), and 181.9 (C-10). The ¹H NMR spectrum showed the presence of two highly de-shielded singlets resonating at δ 11.96 and 12.08 due to the presence of two chelated hydroxyl groups on C-1 and C-8 respectively.

The ¹H NMR spectral pattern of **1** showed a pair of de-shielded meta-coupled protons at δ 7.06 (1H, d, H-2, J=1.4 Hz), and 7.60 (1H, d, H-4, J =1.4 Hz) in addition to methyl at δ 2.44 (3H, s), an ABX spin system was observed for three aromatic protons

resonated at δ 7.79 (1H, dd, H-5, $J=7.7$ & 1.1 Hz), 7.65 (1H, t, H-6, $J=7.7$ Hz), and 7.26 (1H, dd, H-7, $J=7.7$ & 1.1 Hz). The obtained NMR data were consistent with those of chrysophanol.[24]

Compound 2 was obtained as a yellow red amorphous powder and gave on TLC [$R_f = 0.52$, using pre-coated silica gel plate and n.hexane: ethyl acetate (8:2)] deep red spot after fuming with NH_3 vapor indicating an anthraquinone derivative. The Compound **2** shows a pseudo-molecular ion peak at 301 m/e $[\text{M}+\text{H}]^+$ based on the positive ESI/MS analysis, which is consistent with the molecular formula $\text{C}_{16}\text{H}_{12}\text{O}_6$. In the UV spectrum of [2], absorption maxima were observed at 254, 290, 355 and 420 nm, suggestive of an anthraquinone structure. The IR spectrum showed absorption bands at 3455, 1630 and 1680 cm^{-1} suggesting the presence of hydroxyl and free carbonyl functions, respectively.

The ^{13}C NMR, DEPT, and HMQC of Compound **2** displayed 16 carbon signals, including one methyl group at δ 8.05 (on C-3), 3 methines at δ 119.65 (C-5), 136.29 (C-6), 116.37 (C-7), and a methoxy carbon at δ 56.22 (on C-8); and 7 quaternary carbons at δ 153.8 (C-1), 151.59 (C-2), 118.0 (C-3), 160.15 (C-4), 116.7 (C-4a), 135.2 (C-5a), 118.0 (C-8a) and 116.7 (C-1a) as well as two carbonyl carbons at δ 179.8 (C-9), and 185.0 (C-10).

The ^1H NMR spectral pattern of **2** showed methoxy proton at δ 4.03 (3H, s), and methyl at δ 2.05 (3H, s) in addition to an ABX spin system was observed for three aromatic protons resonated at δ 7.81 (1H, d, H-5, $J=8.01$ Hz), 7.71 (1H, t, H-6, $J=8.01$ Hz), and 7.26 (1H, dd, H-7, $J=8.01$). The positions of these proton chemical shifts are established after HMBC analysis. To the best of our knowledge this is the first report for isolation of this compound in nature.

Compound 3 was isolated as a yellowish-white amorphous powder and gave on TLC [$R_f = 0.45$, using pre-coated silica gel plate and n.hexane: ethyl acetate: methanol (6:4:1)] deep yellow spot after spraying with AlCl_3 indicating flavonoid compound. The Compound **3** shows a pseudo-molecular ion peak at 287 m/e $[\text{M}+\text{H}]^+$ based on the positive ESI/MS analysis, which is consistent with the molecular formula $\text{C}_{15}\text{H}_{10}\text{O}_6$. ^1H NMR of compound **3** shows 2

meta-coupled aromatic proton signals at δ 6.18 (d, 2.22 Hz, H-6) and 6.42 (d, 2.22 Hz, H-8). The proton spectrum also shows an ABM spin system at δ 6.87 (d, 8.4 Hz, H-5'), 7.41 (dd, 8.4, 2.2 Hz, H-6') and 7.39 (d, 2.2 Hz, H-2') in addition to a sharp singlet at 6.65 (s, H-3). There is another sharp singlet at 12.95 of 5-OH due to para-hydroxy-effect. NMR data and mass fragmentation pattern are completely identical with those of luteolin. [25]

Compound 4 was obtained as a yellow white amorphous powder, give blue fluorescent light on TLC [$R_f = 0.4$, using pre-coated silica gel plate and n.hexane: ethyl acetate: methanol (6:4:1)]. Not affected with NH_3 vapors, KOH, or AlCl_3 spray reagents. The Compound **4** shows a pseudo-molecular ion peak at 315 m/e $[\text{M}+\text{H}]^+$ based on the positive ESI/MS analysis, which is consistent with the molecular formula $\text{C}_{18}\text{H}_{18}\text{O}_5$.

The ^1H NMR spectral pattern of **4** (DMSO- d_6) showed δ : 6.45 (1H, d, H-2, $J=15.7$ Hz), 7.33 (1H, d, H-3, $J=15.7$ Hz), 7.12 (1H, d, H-2', $J=1.8$ Hz), 6.78 (1H, d, H-5', $J=8.1$ Hz), 6.98 (1H, dd, H-6', $J=8.1$ & 1.8 Hz), 3.35 (1H, t, H-2a'', $J=7.2$ Hz), 3.33 (1H, t, H-2b'', $J=7.2$ Hz), 2.65 (2H, t, H-3'', $J=7.2$ Hz), 7.02 (2H, d, H-2''' & H-6''', $J=8.4$ Hz), 6.68 (2H, d, H-3''' & H-5''', $J=8.4$ Hz) and 3.79 (3H, s, O-Me). The obtained NMR data were consistent with those of *p*-hydroxyphenethyl trans-ferulate.[26]

Compound 5 was obtained as a yellow amorphous powder and gave on TLC [$R_f = 0.52$, using pre-coated silica gel plate and n.hexane: ethyl acetate (8:2)] deep red spot after fuming with NH_3 vapors indicating an anthraquinone derivative. The Compound **5** shows a pseudo-molecular ion peak at 287 m/e $[\text{M}+\text{H}]^+$ based on the positive ESI/MS analysis, which is consistent with the molecular formula $\text{C}_{15}\text{H}_{10}\text{O}_6$.

The ^1H NMR spectral pattern of **5** showed a pair of de-shielded meta-coupled protons at δ 6.56 (1H, d, H-2, $J=2.4$ Hz), and 7.60 (1H, d, H-4, $J=2.4$ Hz) in addition to methyl at δ 2.49 (3H, s), and aromatic proton resonated at δ 7.22 (1H, s, H-5). The obtained NMR data were consistent with those of 7 hydroxy emodin. [27]

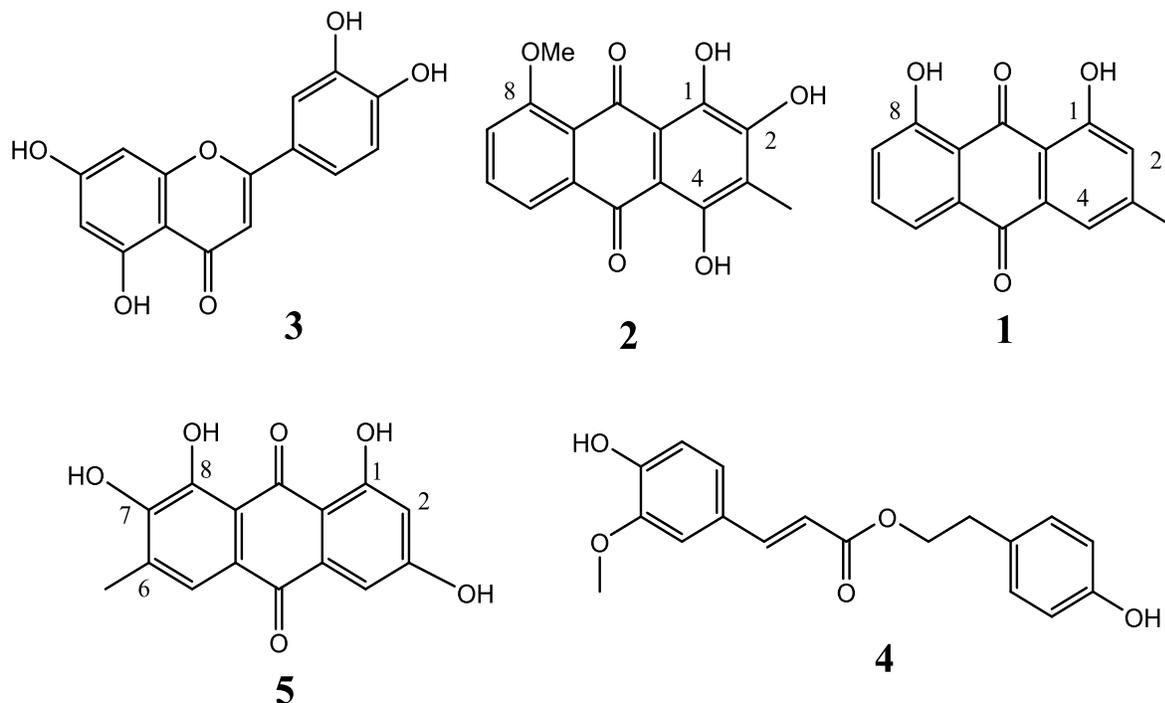


Figure 1: The structures of compounds 1, 2, 3, 4, and 5

Total flavonoid contents

The result of total flavonoid experiment was calculated as 30 mg rutin equivalents/1gram using the regression equation obtained from the standard calibration curve.

Total polyphenolic contents

The results of total polyphenolic Experiment was calculated as 52 mg gallic acid equivalent/1gram using the regression equation obtained from the standard calibration.

In present study, the doses of 200 mg/kg *Extract* caused significant ($P < 0.05$) Increase in mean latency after 30, 60, 90 min compared to negative control but not comparable with the analgesic activity of standard drug.

The antimicrobial activity

The in vitro studies in this work of all five fractions i.e. total extract, hexane, ethyl acetate, chloroform and n-butanol fractions were carried out against pathogenic bacterial and fungal strains. If 0.2 mg/mL concentration of test sample produce 6 mm zone against clinically pathogenic organism then it is considered effective. All fractions except ethyl acetate fractions were found ineffective. The zones of inhibition measured 15 ± 0.11 mm, 11.0 ± 0.10 mm, 14.0 ± 0.50 mm, and 15.0 ± 0.20 mm respectively against bacteria. The standard drug Gentamycin

formed 12.0 ± 0.30 mm of zone against *E. coli* and 10.0 ± 0.10 mm against *k. pneumonia*. Both of the above mentioned organisms are Gram negative but the result suggests use for both infections. As far as Gram positive organisms are concerned, good antibacterial activity was found with 11.0 ± 0.60 mm zone inhibition against *S. aureus* and 15.0 ± 0.40 mm against *B. Subtilis*, while the standard drug Gentamycin formed 12.0 ± 0.10 mm and 8.40 ± 0.40 mm zones of inhibition, respectively.

Antifungal activity was screened for *Candida albicans* which exhibited mild positive activity for ethyl acetate fraction only. The size of zones was found to be 23.10 ± 0.05 mm and against standard drug coltiramazole formed 35.10 ± 0.20 mm zone of inhibition against *C. albicans*.

Toxicity Study

The animals were observed continuously for 30 minutes and 24 hours for behavioral and physiological toxic effects at the dose of 1000 up to 6000mg/kg orally.

The lethality and toxic signs were not detected and the extract produced no mortality up to 6000 mg/kg.

The antioxidant activity

There are significant variations in the capacity of total extract, hexane, ethyl acetate, chloroform and n-butanol fractions to scavenge the DPPH radical with IC_{50} ranging from 6.9 to 23 μ g/mL. From the

estimated IC_{50} values, the order of potency is *ethyl acetate* extract with IC_{50} 6.9 $\mu\text{g/mL}$, *n*-butanol extract with IC_{50} 15.6 $\mu\text{g/mL}$, total extract with IC_{50} 18.1 $\mu\text{g/mL}$ followed by chloroform extract with IC_{50} 20.2 $\mu\text{g/mL}$. The ethyl acetate and *n*-butanol extracts showed a higher DPPH scavenging activity superior to that of the positive control (ascorbic acid 14.2 $\mu\text{g/mL}$).

The cytotoxic activity

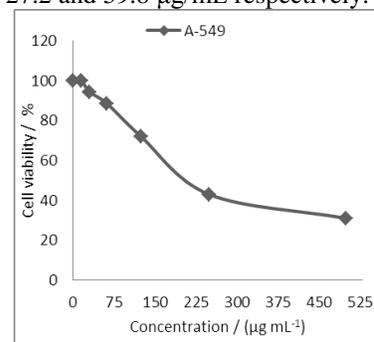
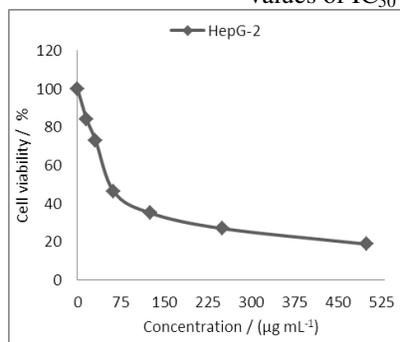
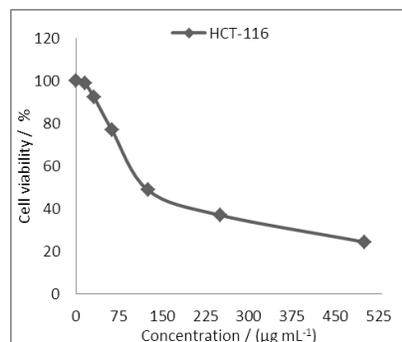


Figure2: Cytotoxic activity of total extract against cancer cell lines HCT-116, hepatoma carcinoma cells-2 and A-549 respectively

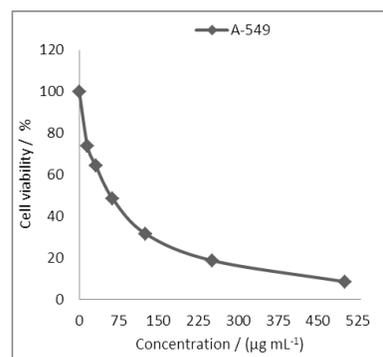
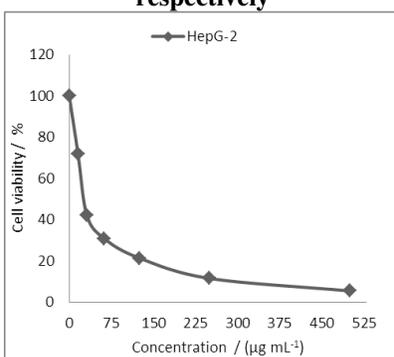
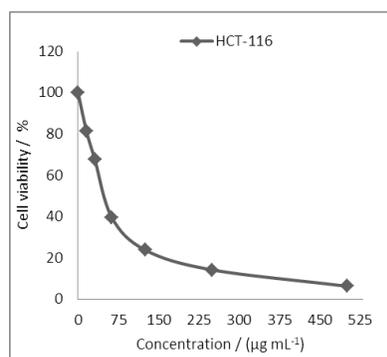


Figure3: Cytotoxic activity of Ethyl acetate extract against cancer cell lines HCT-116, hepatoma carcinoma cells-2 and A-549 respectively.

The analgesic activity

In hot-plate model, the total extract showed significant analgesic activity by increasing the reaction time of the mice compared to negative control. Diclofenac Sodium was used as positive control drug which was considered as mild analgesic activity. In comparison with negative control 200 ml/kg has shown most significant at $P < 0.05$ during

60 min and 90 min observation, while 300 ml/kg showed less significant effect in same time Figure 4.

The statistical analysis of data was performed using one-way ANOVA on result data. Multiple Comparisons with Tukey-tests were also performed. The mean, standard error of means and analysis of variance (ANOVA) were calculated by computerized programs SPSS 15.

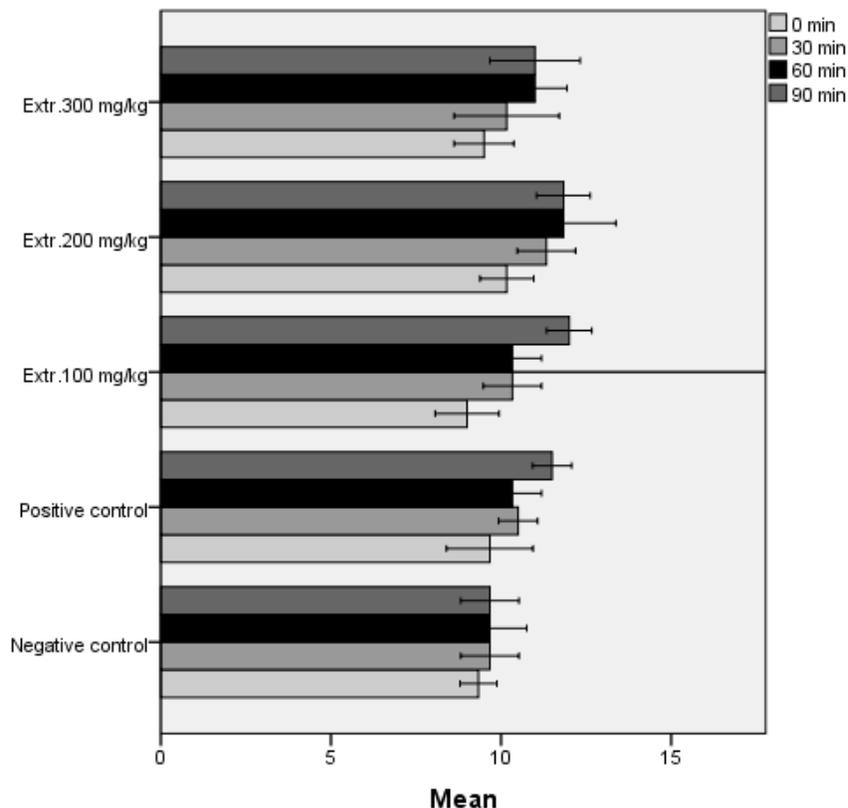


Figure 4: Effect of extract on latency to hot plate test in mice

CONCLUSIONS:

It was suggested that further work should be carried out to isolate, purify, and characterize more active constituents responsible for the activity of these extracts. Also, additional work is encouraged to elucidate the possible mechanism of action of these constituents.

The study was approved by the Institutional Ethics Committee.

ACKNOWLEDGEMENT:

The authors are thankful to support of this research study by the grant No. PHM-70066 - 2017-1-7-F from Deanship of scientific research at Northern Border University, K.S.A.

The authors are also thankful to Department of Pharmacology and Toxicology for provided good animal for project.

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