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Determination of antioxidant and bimolecular protective effect of *Berberis calliobotrys* Aitch. ex Koehne extracts and essential oil against H_2O_2 induced oxidative damage to *p*BR 322 DNA and RBCs

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Abstract: We investigated the protective effect of fractions and essential oil from *Berberis calliobotrys* on H_2O_2 induced oxidative damage on pBR322 DNA. The crude plant material was extracted using 90% methanolic and liquid-liquid fractionation was accomplished. The essential oil analysis was performed using GC/MS. The FRAP and DPPH assays were performed to determine antioxidant activity. The DNA protection assay was performed using plasmid pBR 322 DNA. The essential oil analysis indicated presence of germacrene D (9.26%), stearic acid (7.50%), methyl tetradecanoate (6.36%) *α*-thujene (5.71%) and *α*-muurolol (5.30%) methyl eugenol (5.17%). *In vitro* analysis showed significant antioxidant activity of all tested extracts and essential oil. The extract showed significant effects at (1000 µg/mL) on pBR322 DNA. Finally it was concluded that *Berberis calliobotrys* possesses signifgant protective on effects pBR322 DNA and RBC cellular membrane.

Keywords: Traditional usage, oxidative damage, essential oils, pBR322 DNA, biomarkers.

INTRODUCTION

Free radicals are the unpaired electron-containing molecules, that are capable to get stabilized by scavenging electrons from other sources. Free radicals and reactive oxygen species (ROS) have been involved in harming the DNA by generating oxidized forms of purine and pyrimidine bases causing the breakage of a single strand (ss) of DNA. The oxidation-mediated damage of macromolecules like DNA, proteins, and lipids leads to various pathological conditions including cancer and aging. A typical example of ROS is hydrogen peroxide (H_2O_2), which has been involved in the synthesis of hydroxyl radicals leading to DNA damage (Ransy *et al*., 2020). Various valuable compounds, known as anti-oxidants, control the formation of free radicals resulting in the prevention of oxidative processes (Santos-Sanches *et al*., 2019). Eating foods that have plentiful of phytochemicals like phenolics and flavonoids have been linked to a decreased risk of cancer due to anti-oxidant property. It has renewed the attention towards diets rich in plants (Rodríguez-García *et al.,* 2019).

Berberis calliobotrys Aitch. ex Koehne (Berberidaceae), commonly known as Choweng, is an important medicinal plant mostly found in North Temperate regions, tropical mountains and South America (Ali and Nasir, 1989). This plant is used in various systems of medicines for treating different types of ailments. The fruits of the plant are crushed and boiled in water and used for fever. A paste is prepared from the bark of the rhizome by mixing with equal weight of black pepper and butter and is applied locally for backache. Decoction of bark is used as a gargle for pharyngitis. This plant is also used in some liver infections and intestinal colitis (Ali and Qaiser, 2009). An aporphine - benzylisquinoline alkaloid, khyberine has been reported in *Berberis calliobotrys* (*B. calliobotrys*) in about one part per million. Other alkaloids which were obtained and characterized from the plant include Pakistanamine, Methylpakistanine, Pakistanine, Chitraline and Kalashine (Salehi *et al*., 2019).

The extensive folklore uses of *B. calliobotrys* for the treatment of different diseases has led to ensure its antioxidant potential. It is revealed from literature survey that *B. calliobotrys* has not been evaluated for antioxidant property till to date. In the present study, antioxidant potential and safety profile of this plant specie was evaluated which might be utilized to standardize the plant for therapeutic purposes.

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MATERIALS AND METHODS

Collection and preparation of plant material

The hilly areas of Quetta, Balochistan, (Pakistan) were focused for plant collection. The specimen were authenticated by Taxonomist and specimen were deposited (Sultan Ayub Herbarium, GC University Lahore, No.1912). The plant was dried in sun shade and pulverized. The powdered plant material was extracted with methanol and rotary evaporator was used to concentrate and dry the plant material. Finally the plant material was stored in a refrigerator at 4°C, till further used in experiments.

Essential oil (EO) Isolation

The powdered plant material was processed for hydro-distillation for 4h (three times) by using a Clevenger-type apparatus (Elyemni *et al*., 2019). The percentage yield of EO was determined. The collected EO was stored till further usage.

GC-MS analysis

The GC/MS system (GC 6850) was equipped with auto injector (7683B series) and inert mass detector (No.5973; Agilent ® DE, USA). The HP-5 MS capillary column (30.0 m × 0.25 mm, film thickness 0.25µm) was employed and helium gas was used as mobile phase. The constituents were examined in scanning mode using 45-550 *m/z* in GC/MS. The retention index (RI) of components were compared with standard compounds and further authentication was accomplished using MS data with NIST 0.5 (Mass Spectral Library) (Adams, 2011).

Determination of total phenolic contents (TPC)

The phenolic contents of tested samples were evaluated by previously described method (Phuyal *et al*., 2020). Briefly, Folin- Ciocalteu's reagent (0.1mL) was mixed with sodium carbonate (10%; 2.8mL) and test sample (0.5 mg/mL) and kept untouched for 40 min. Afterwards, the absorbance was recorded at 725 nm by using UV spectrophotometer. Results were expressed in GAE mg/g.

Antioxidant activity by DPPH scavenging assay

Briefly, in 96 microwells plate, the test sample (10µL) was mixed with DPPH solution (90µL; 100µM) and plates were placed in dark for 30 min (37°C). After incubation, absorbance was carried out at 517 nm to check out reduction of sample by using a micro plate reader. Ascorbic acid was taken as the standard (Kumar *et al*., 2020). The % inhibition was calculated as

Inhibition (%) = 100 - (absorbance of test sample/ absorbance of control) × 100

Ferric Reducing Antioxidant Power assay (FRAP)

The reducing power assay was performed using standard protocol with slight modifications (Spiegel *et al.*, 2020) was used to investigate the antioxidant potential. During this experiment, various concentrations of test sample and standard (ascorbic acid) were prepared and mixed thoroughly with sodium phosphate buffer at pH 6.6 (2.5 mL; 200 mM) and potassium ferricyanide (1%; 2.5mL) followed by incubation at 50°C for 20 min. Afterwards, trochloroacetic acid (10% TCA; 2.5mL) was added and centrifuged (3000 rpm; 5 min). Later, aliquot of 2.5mL of the supernatant (upper layer) was mixed with 2.5mL of distilled water and ferric chloride (0.1%; 0.5mL) and the absorbance was measured at 700 nm. Reducing power was determined after plotting optical density against concentrations of test sample.

DNA protection assay

DNA protection assay was carried out by the reported method with minor modifications (Kalpana *et al*., 2009). In this assay plasmid pBR 322 DNA with H_2O_2 and UV light was used as a control. The reaction mixture was prepared by diluting plasmid pBR 322 DNA (0.5 µg) with 50mM sodium phosphate buffer (pH 7.4; 3µL). Afterwards diluted pBR 322 DNA (3µL) was added to test sample (5 µL.) After this 30% H_2O_2 (4µL) was added and volume was made up to 15µL (Using PBS pH 7.4) followed by incubation at in dark at 37°C for one hour. After incubation the plasmid DNA was loaded on agarose gel (1%) and heated in a microwave oven to get a homogeneous mixture. Once a suitable temperature was achieved, ethidium bromide (20µL) was added to agarose and allowed to solidify for 20 min. The reaction was run on an electrophoresis assembly (horizontal at 100 Volts for 1 hr; Bio-Rad Singapore). The comparative difference in migration between oxidized and native DNA was observed.

RBC cellular membrane protection assay

The cytotoxicity of the essential oil was analyzed by the hemolytic method (Aslam *et al.,* 2011) with minor modifications. The heparinized human blood (3mL) was centrifuged for 5 min and supernatant obtained. The RBCs were cleaned using phosphate-buffered saline (5mL) solution and cell counting was performed on haemacytometer. The erythrocyte count was retained to 7.068×10^8 cells/mL. An aliquot test sample (20 µL) were added to diluted RBCs suspension (180µL). Using Triton X-100 (0.1%) was used as positive control samples were incubated at 37°C for 35 min. Afterwards; the tubes were instantaneously positioned on ice for 5 min and centrifuged (5 min). The supernatant (100µL) was further diluted (900 µL) followed by addition of 200µL mixture from each tube to 96 micro well plates. Lastly, the absorbance was recorded at 576 nm (BioTeck, Winooski, VT, USA). The RBCs lysis (%) was calculated by the following formula:

Lysis of RBCs (%) = (absorbance of test sample / absorbance of control) × 100

Table 1: Chemical compounds in essential oil of *B. calliobotrys* analysed by GCMS

S. No	Retention Index (RI)	Chemical compounds	Area (% Conc)
1	933	*α*-thujene	5.71
2	940	*α*-pinene	1.23
3	953	camphene	1.76
4	970	*β*-pinene	1.64
5	979	*β*-myrcene	0.69
6	1025	*p*-cymene	4.37
7	1028	limonene	1.83
8	1030	1,8-cineole	1.53
9	1033	*β*-phellandrene	0.66
10	1049	*β*-ocirmene	2.84
11	1058	*γ*-terpinene	2.36
12	1098	linalool	0.68
13	1138	carveol	3.02
14	1204	verbenone	3.63
15	1291	thymol	1.06
16	1293	2-undecanone	5.26
17	1297	carvacrol	0.92
18	1361	eugenol	0.94
19	1392	β-elemene	0.62
20	1411	methyl eugenol	5.17
21	1411	*α*-copaene	3.36
22	1417	*β*-caryophyllene	0.89
23	1439	*α*-guaiene	1.05
24	1451	*α*-humulene	3.27
25	1483	germacrene D	9.26
26	1493	valencene	1.75
27	1513	*γ*-cadinene	0.63
28	1577	spathulenol	1.74
29	1647	*α*-muurolol	5.30
30	1654	*α*-cadinol	1.08
31	1719	methyl tetradecanoate	6.36
32	1962	hexadecanoic acid methyl ester	1.49
33	2000	eicosane	0.71
34	2124	octadecanoic acid methyl ester	5.22
35	2173	3-methylheneicosane	1.14
36	2194	octadecanoic acid ethyl ester	1.58
37	2200	stearic acid	7.50

Mode of identification= Retention Index (RI) and comparison of mass spectra

Table 2: DPPH scavenging activity of *B. calliobotrys*

S. No	Sample	Concentration (mg/mL)	% inhibition	IC_{50} (mg/mL)
1	Methanol	5	60 ± 0.70	0.0038 ± 0.69[a]
2	*n*-butanol	5	88 ± 0.07	0.0021 ± 1.17
3	Ethyl acetate	5	90 ± 0.11	0.00092 ± 0.91
4	Chloroform	5	73 ± 1.80	0.0034 ± 0.31
5	*n*-hexane	5	84 ± 3.60	0.0017 ± 0.78
6	Essential oil	5	85± 0.54	0.0024 ± 1.02
7	Vitamin C[b]	0.5mM	90 ± 0.11	0.00097 ± 1.14

[a]Results are presented as mean ± standard mean error of three assays (p<0.05)
[b]Standard antioxidant

STATISTICAL ANALYSIS

A two way ANOVA analysis performed by using SPSS version. 13.0 and results were presented as ± SD.

RESULTS

GCMS analysis

The GC/MS analysis of the EO showed presence of series of compounds (table 1) with a diversified chromatograms (fig. 1). The chief chemical elements established in the essential oil of the plant were germacrene-D (9.26%), stearic acid (7.50%), methyl tetradecanoate (6.36%), *α*-thugene (5.71%), *α*-muurolol (5.30%), undecanone (5.26%), octadecanoic acid methyl ester (5.22%) and methyl eugenol (5.17%) (table 1; fig. 2). The percentage yield of essential oil obtained from *B. calliobotrys* was 0.31% (w/w of dry plant material).

Table 3: Hemolysis (%) caused by *B. calliobotrys*

Extract and fractions	% Hemolysis
Methanol	8.96±0.04[a]
n-butanol	6.32±0.04
Ethyl acetate	4.80±0.07
Chloroform	2.27±0.04
n-hexane	3.05±0.04
Essential oil	2.09±0.03
Triton X-100[b]	99.69±0.60

[a] Results are presented as mean ± standard mean error (p<0.05)
[b] Standard

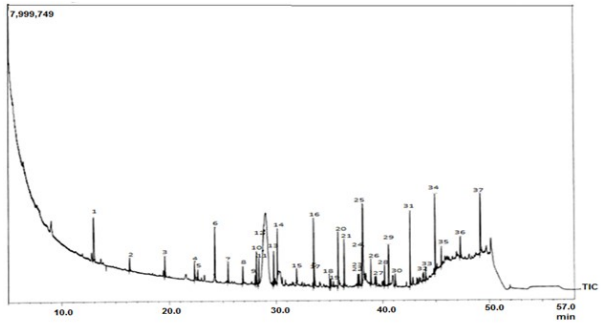


Fig. 1: The GC-MS chromatogram of *B. calliobotrys* essential oil

Antioxidant activity

The DPPH scavenging assay was employed to determine the antioxidant potential test samples. The ethyl acetate fraction presented significant antioxidant activity (IC_{50} 0.92µg/mL), followed by *n*-hexane (IC_{50} 0.92µg/mL) and *n*-butanolic (IC_{50} 2.1µg/mL) fraction. Likewise, the essential oil showed a high antioxidant activity (IC_{50} 2.4 µg/mL) (table 2). For ferrous reducing power (FRAP), the methanol extract showed maximum antioxidant activity at all the concentrations compared to all other fractions. The ethyl acetate, *n*-butanol fractions and essential oil showed comparable antioxidant activity (fig. 3). The *n*-hexane fraction proved to be a poor antioxidant.

Total phenolic contents (TPC)

The total phenolic contents were estimated in plant extract and essential oil and high values were recorded (fig. 4).

DNA protection effect

Based on interesting results of antioxidant potential of crude extract and essential oil, the DNA protection assay was performed to ascertain their safety. The results showed the modifications in the extent of fortification of plasmid *p*BR322 DNA by H_2O_2 tempted injury; upon treatment with test samples (fig. 5).

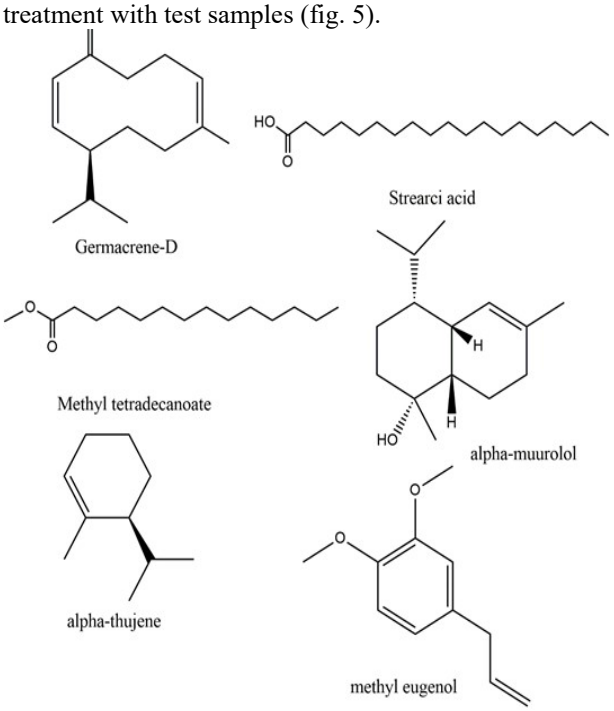


Fig. 2: Major compound identified by GCMS.

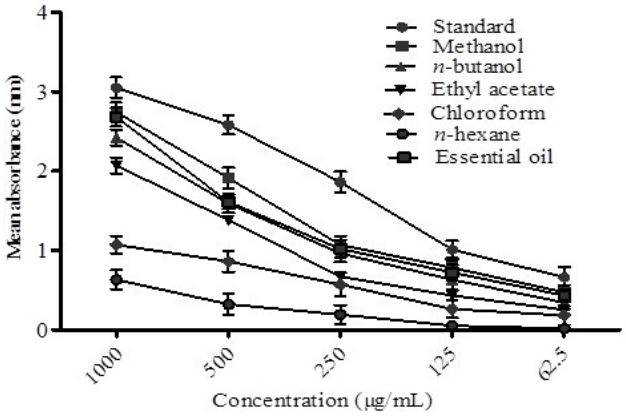


Fig. 3: Reducing power by *B. calliobotrys* extract, fractions and essential oil (p<0.05)

RBC cellular membrane protection effect

To evaluate the cytotoxicity of test samples, hemolytic activity method was used based on human red blood cells (RBCs). The highest hemolytic effect was shown by methanol extract (8.96%) followed by the *n*-butanol fraction (6.32%), Ethyl acetate (4.80%), *n*-hexane fraction

(3.05%) and chloroform (2.27%). The essential oil showed the least hemolytic effect and based on literature, it was concluded the plant extracts and fractions may be safe for human use (table 3).

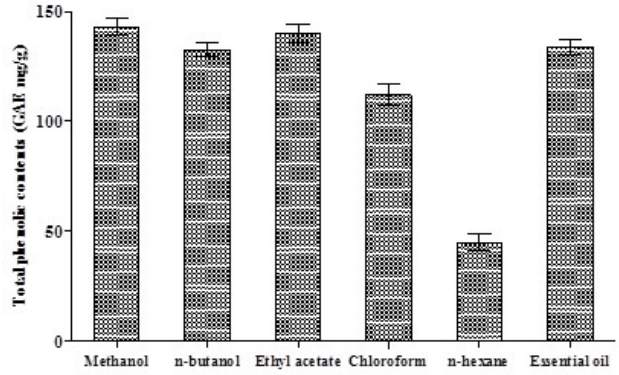
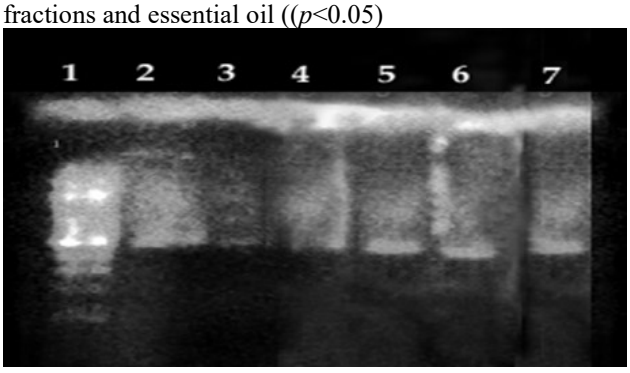


Fig. 4: Total phenolic contents by *B. calliobotrys* extract, fractions and essential oil (p<0.05)



(Lane 1 = Plasmid pBR322 DNA without treatment (Super coiled); Lane 2 = Plasmid pBR322 DNA and dye), Lane 3 = Plasmid pBR322 DNA; treated with H_2O_2 (open circular or damaged); Lane 4 = Plasmid pBR322 DNA treated with methanol extract + H_2O_2; Lane 5 = Plasmid pBR322 DNA treated with *n*-butanol fraction + H_2O_2; Lane 6 = Plasmid pBR322 DNA treated with ethyl acetate fraction + H_2O_2; Lane 7 = Plasmid pBR322 DNA treated with essential oil + H_2O_2)

Fig. 5: Electropherogram showing DNA protection effect by *B. calliobotrys* extract, various organic fractions and essential oil with H_2O_2 induced oxidative damage on pBR322 DNA.

DISCUSSION

The antioxidant potential of methanol extracts was due to the occurrence of phenolics, flavonoids and other phytochemicals (Lecumberri *et al*., 2007) The reduction in the number of DPPH molecules can be ascribed to the presence of OH groups in the tested samples (Bhaskar and Balakrishnan, 2009). The basic standard of the DPPH assay is transformation of color (purple to yellow) as the radical is extinguished by antioxidants (Kahkonen *et al*., 1999). The extent of discoloration indicated the scavenging properties of plant extract and its fractions (Jayaprakasha *et al*., 2007). As per earlier reports antioxidants donate protons to DPPH free radicals, the absorption decreased with increase of concentration of the test sample. This decrease in absorption was employed to assess the extent of antioxidant to scavenge free radicals (Turkoglu *et al*., 2007). The least value of IC_{50} represented a better antioxidant and greater value for less antioxidant potential.

The phenolics have been described as to remove the free radicals due to the existence of the hydroxyl group. The phenolic compounds have a significant role in the maintenance of lipid and fat oxidation. The polyphenols also have been linked with antioxidant activity (Cakir *et al*., 2003) and the phenolic compounds might have the inhibitory effects on carcinogenesis and mutagenesis in human beings when taken from a food such as vegetables and fruits (George and Rupasinghe, 2017). It was also revealed from the literature that the phenolic contents of the plants varied with the nature of organic solvents resembling with our findings. Since our tested fractions and essential oil has high phenolic contents, promising antioxidant activities were recorded. Further, earlier reports showed that the phenolic compounds present in various plant extracts play a vital role in their antimicrobial, antioxidant and other activities (Bouarab-Chibane *et al*., 2020). The antioxidant potential of essential oils is due to the presence of phyto constituents. It has been reported earlier that limonene; β-pinene; 1,8-cineole; germacrene; α-pinene; pulegone; (Derwich *et al*., 2011, Valdivieso-Ugarte *et al*., 2019) and thymol (Escobar et al., 2020) found in essential oils posess antioxidant properties

During DNA protection assay, it was observed that the DNA was protected from nicking as treated with extracts and essential oil. When the plasmid was exposed to H_2O_2, the DNA damage was evident (third lane) (fig. 4). The *n*-butanol, ethyl acetate part and EO (5^{th}, 6^{th} and 7^{th} lane) displayed a protecting influence and the DNA band was protected from damage by H_2O_2. The methanolic part exhibited a less protective result on DNA (fourth lane) with comparably lesser protection compared to other fractions and essential oil when compared with the other lanes. The highest effective DNA protection was shown by *n*-butanol, ethyl acetate parts and E.O of *B. calliobotrys* and the lowest by the methanol extract. The protective effect of the samples might be due to the presence of phenolic and flavonoids present in them and also their ability to scavenge the free radicals and oxidation products (Salar *et al*., 2017; Mansoori *et al*., 2020). The hydroxyl radicals have a very short lifetime than other free radicals and these radicals outbreaks the DNA double strands converting them into single strand i.e. open circular form (Elmegerhi *et al*., 2018). The phytochemicals such as phenolics and flavonoids play an imperative part in quenching the free radicals and thus lessen the DNA damage (George and Rupasinghe, 2017; Wu and Dhanasekaran, 2020).

The toxicity profiling of the samples were determined using RBC cellular membrane protection effect. The mechanical stability of the red blood cell membrane is essential to reduce the cytotoxic effects of different compounds. The least *in vitro* cytotoxicity of different plant extracts is of great importance for their safe use in different diseases other than cancer (Uddin *et al*., 2014). In the present research all the samples tested for *in vitro* hemolytic activity were found in a safe range, i.e. below 10.0%, so it could be expected that the methanol extract and is various organic fractions have a minor cytotoxicity. The plant extracts or fractions having minor cytotoxicity might be used as herbal medicines (Aslam *et al*., 2011).

CONCLUSION

It was concluded that both extract and essential oil of *B. calliobotrys* possess potential protective effects on DNA and RBC cell membrane from damage by oxidative stress.

REFERENCES

Adams RP (2001). Identification of essential oil components by gas chromatography - mass pectroscopy. Allured Publishing Corporation, USA, pp.1-804.

Ali H and Qaiser M (2009). The ethnobotany of Chitral valley, Pakistan with particular reference to medicinal plants. *Pak. J. Bot.,* **41**(4): 2009-2041.

Ali SI and Nasir E (1989). Flora of Pakistan. Department of Botany, University of Karachi: Karachi. p.1-145.

Aslam F, Rasool N, Riaz M, Zubair M, Rizwan K, Abbas M, Bukhari TH, Bukhari I H (2011). Antioxidant, haemolytic activities and GC-MS profiling of *Carissa carandas*. *Int. J. Phytomed.,* **3** (4): 567-578.

Bhaskar HV and Balakrishnan N (2009). *In vitro* antioxidant property of Laticiferous plant species from Western Ghats Tamilnadu, India. *Int. J. Health Res.,* **2**(2): 163-170.

Bouarab-Chibane L, Forquet V, Lanteri P, Clément Y, Léonard-Akkari L, Oulahal N, Degraeve P and Bordes C (2019). Antibacterial Properties of Polyphenols: Characterization and QSAR (Quantitative Structure-Activity Relationship) Models. *Front. Microbiol.,* **10**: 829.

Cakir A, Ahmet YMA, Harmandar M and Kazaz C (2003). Isolation and characterization of antioxidant phenolic compounds from the aerial parts of *Hypericum hyssopifolium* L. by activity-guided fractionation. *J. Ethnopharmacol.,* **87**(1): 73-83.

Derwich E, Benziane Z, Chabir R and Taouil R (2011). Characterization of volatiles and evaluation of antioxidant activity of the flower essential oils of *Myrtus communis* L. from Morocco. *Int. J. Current Pharm. Res*., **3**(3): 17-23.

Elmegerhi S, Su C, Buglewicz DJ, Aizawa Y, Kato TA (2018). Effect of hydroxyl group position in flavonoids on inducing single-stranded DNA damage mediated by cupric ions. *Int. J. Mol. Med.,* **42**(1): 658-664.

Elyemni, M, Louaste B, Nechad I, Elkamli T, Bouia A, Taleb M, Chaouch M and Eloutassi N (2019). Extraction of essential oils of *Rosmarinus officinalis* L. by two different methods: Hydro distillation and microwave assisted hydro distillation. *Sci. World J*. 2019: Article ID 3659432.

George VC and Rupasinghe H (2017). Apple flavonoids suppress carcinogen-induced DNA damage in normal human bronchial epithelial cells. *Oxid. Med. Cell. Longev*., 21767198.

Escobar, A., Perez M., Ramanelli and Blustein G (2020). Thymol bioactivity: A review focusing on practical applications. *Arab. J. Chem*., **13**(12): 9243-9269.

Jayaprakasha GK, Negi PS, Jena BS and Rao LJ (2007). Antioxidant and antimutagenic activities of *Cinnamomum zeylanicum* fruit extracts. *J. Food Comp. Anal.,* **20**(3-4): 330-336.

Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS and Heinonen M (1999). The antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food. Chem.,* **47**(10): 3954-3962.

Kalpana KB, Srinivasan M and Menon VP (2009). Evaluation of antioxidant activity of hesperidin and its protective effect on H_2O_2 induced oxidative damage on pBR322 DNA and RBC cellular membrane. *Mol. Cell. Biochem.,* **323**(1-2): 21-29.

Kumar J, Kumar N, Sati N and Hota PK (2020). Antioxidant properties of ethenyl indole: DPPH assay and TDDFT studies. *New J. Chem.* **44**(21): 8960-8970.

Lecumberri E, Mateos R, Izquierdo-Pulido M, Ruperez P, Goya L and Bravo L (2007). Dietary fibre composition, antioxidant capacity and physico-chemical properties of a fibre rich product of cocoa (*Theobroma cacao* L.). *Food. Chem.,* **104**(3): 948-954.

Mansoori A, Singh N, Dubey SK, Thakur TK, Alkan N, Das SN and Kumar A (2020) Phytochemical characterization and assessment of crude extracts from *Lantana camara* L. for antioxidant and antimicrobial activity. *Front. Agron.,* **2**: 582268.

Rodriguez-Garcia C, Sanchez-Quesada C and J Gaforio (2019). Dietary flavonoids as cancer chemo preventive agents: An updated review of human studies. *Antioxidants* (Basel). **8**(5): 137.

Phuyal N, Jha PK, Raturi PP and Rajbhandary S (2020). Total phenolic, flavonoid contents and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. *Sci. World J.* **2020**: 8780704.

Ransy C, Vaz C, Lombes A and Bouillaud F (2020). Use of H_2O_2 to cause oxidative stress, the catalase issue. *Int. J. Mol. Sci*., **21**(23): 9149.

Santos-Sanches NF, Salas-Coronado R, Villanueva-Canongo C and Hernández-Carlos B (2019). Antioxidant compounds and their antioxidant mechanism. *In*: (Ed.), Antioxidants. IntechOpen.

Salar RK, Purewal SS and Sandhu KS (2017). Relationships between DNA damage protection activity, total phenolic content, condensed tannin content and antioxidant potential among Indian barley cultivars. *Biocatal. Agric. Biotechnol*., **11**(2): 201-206.

Salehi B, Selamoglu Z, Sener B, Kilic M, Kumar Jugran A, de Tommasi N, Sinisgalli C, Milella L, Rajkovic J, Flaviana B, Morais-Braga M, F Bezerra C, E Rocha J, D M Coutinho H, Oluwaseun Ademiluyi A, Khan Shinwari Z, Ahmad Jan S, Erol E, Ali Z, Adrian Ostrander E, Sharifi-Rad J, de la Luz Cadiz-Gurrea M, Taheri Y, Martorell M, Segura-Carretero A and Cho WC (2019). *Berberis* plants-drifting from farm to food applications, phytotherapy and phyto-pharmacology. *Foods*, **8**(10): 522.

Spiegel M, Kapusta K, Kołodziejczyk W, Saloni J, Bikowska BZ, Hill GA and Sroka Z (2020). Antioxidant activity of selected phenolic acids-ferric reducing antioxidant power assay and QSAR analysis of the structural features. *Molecules*., **25**(13): 3088.

Turkoglu A, Duru ME and Mercan N (2007). Antioxidant and antimicrobial activity of *Russula delica* Fr: An edidle wild mushroom. *EJAC.,* **2**(1): 54-67.

Uddin SJ, Grice ID and Tiralongo E (2009). Cytotoxic effects of Bangladeshi medicinal plant extracts. *Evid. Based. Complement. Alternat. Med.,* **2011**: 578092.

Valdivieso-Ugarte M, Gomez-Llorente C, Plaza-Díaz J, Gil A (2019). Antimicrobial, antioxidant, and immunomodulatory properties of essential oils: A systematic review. *Nutrients.*, **11**(11): 2786.

Wu X and Dhanasekaran S (2020). Protective effect of leaf extract of *Abutilon indicum* on DNA damage and peripheral blood lymphocytes in combating the oxidative stress. *Saudi Pharm. J.,* **28**(8): 943-9950.