Evaluation of antioxidative and biological activity of *Houttuynia cordata* extracts

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Abstract- The present study evaluated the antioxidative and biological activity of the aqueous, ethanolic and methanolic extract from leaf and root of aromatic herb, *Houttuyniacordata* (HC). The yield were found to be 7.67±0.33 to 9.33±0.58 %, 9.00±0.58 to 10.67±0.33 % and 11.33±0.33 to 13.67±0.58 % of root to leaf in different solvent of aqueous, ethanolic and methanolic solvent extraction. The parameters such as 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, total phenol contents (TPC), ferric reducing antioxidant power assay (FRAP), superoxide anion radical scavenging activity (NTZ), haemolytic assay (HA) and genoprotective assay were analysed. The results showed the highest radical scavenging (DPPH) effect in the methanolicleaf extract (75.30±2.16 %) and the lowest in the aqueous root extract (28.69±1.03 %). TPC and FRAP values followed similar trend with highest response found in methanolicleaf extract (305.68±0.58 mg g^{-1}), (20.49±0.01mg g^{-1}) and lowest in aqueous root extract (66.68±0.43), (5.17±0.13) respectively. The highest activity for NTZ was estimated in aqueous root extract (92.92±0.90 %) and lowest in methanolic leaf extract (80.96±0.80 %). The haemolytic activity showed the highest effect in aqueous leaf extract (65.62±0.58 %) and lowest in methanolicleaf extracts (27.77±0.18 %). In genoprotective assay, the percentage protection against oxidative damage to DNA found highest in aqueous root extract (55.37±1.53%) and lowest in methanolic leaf extract (30.34±0.89 %). Overall results from present study demonstrated leaf and root extracts of HC extracted with methanolic, ethanolic and aqueous solvents possess excellent antioxidant properties; however, the highest extraction yield and antioxidant responses for the most of the parameters were exhibited in the methanolic extracts of HC than those of ethanolic and aqueous HC extracts.

Index Terms- *Houttuyniacordata*; aqueous, ethanolic and methanolic extracts; free radical; antioxidant; DNA damage.

I. INTRODUCTION

Free radicals are highly reactive, unstable molecules carries an unpaired number of electrons. It formed constantly in the cells which are potentially dangerous to cells because it regularly steals the electrons from the stable molecules of the cells including DNA, proteins, etc. and make them unstable through the process called oxidation [75]. Therefore, free radicals are toxic to the cells and must be converted into non-toxic form [19]. To neutralize the toxic effects of free radicals or to make free radicals become stable, antioxidants play a main role via inhibiting the oxidation process by donating their own electron [16]. Free radicals are no more dangerous to cells once it gained the electrons from the antioxidants [17]. As a natural defense mechanism, all living organisms contain antioxidant molecules to hunt the free radicals produced in the body. Nevertheless, they are not enough to scavenge those [3] as over-production of free radicals often damages several essential biomolecules such as cellular proteins, membrane lipids, lipoproteins; DNA strands, and thereby disrupts the cellular functions of the cells [3]. Earlier reports proved that antioxidants minimize the risk of chronic diseases including cancer and heart diseases [69]. For these reasons, pharmaceuticals industry serves the antioxidants from natural and synthetic sources for human beings, terrestrial animals and aquatic animals. But an experimental study confirms that synthetic antioxidants cause lipid alteration and carcinogenic effects to the cells [26]. Therefore, the focus of most research has been directed on the development of natural antioxidants from the natural sources of herbal and plant material [75, 62, 15].

For the last several years there has been a growing interest in the exploration /or extraction of antioxidants from many plants and herbs. For more than 8000 years, herbal and plant extracts have been identified as a potential medicine to treat variety of human diseases [20]. It is worth mentioning to note that about 80% of the world's human population during 1980's was solely relied on herbal and plants medicine as a primary source of medicine [21]. Previous studies with secondary metabolites of plants and herbs has provided ample support for the assertion that they contain the active compounds such as peptides, unsaturated long chain fatty acids, aldehydes, flavonoid, alkaloids, essential oils, phenols and water or ethanol soluble, which are used in the therapeutic application against the various human and animal pathogens[52,33,46]. Report says that taking certain taking certain plant/herbal phenolic in the daily diet protect the cells against cardiovascular diseases [66] and cancer [20]. It is also supported that plant/herbal extracts carries antioxidants such as phenolic compounds, flavonoids and tannins that can play an important role in the scavenging free radicals [55, 21**,** 11].

The flowering and perennial herbal plant, *Houttuyniacordata*(HC) belongs to the family of Saururaceae, which is found in different countries including China, Japan, Korea, Thailand, Vietnam and India. *H. cordata* commonly grows in the hilly areas of moist and shady places with an altitude of 300-2600m[7**].**

The vernacular name of *H. cordata* for the different countries is as follows: Yu-Xing-Cao, and Chou-Xing-Cao (QuanzhouBencao) in China; dokudami in Japan; E-Sung-Cho in Korea; Khao-tong or Plu-khao in Thailand; giấpcá or diếpcá in Vietnam and Hangya, Amuli in Arunachal Pradesh, Indiawhere the sample was collected. *H. cordata* has a long history of usage among the tribal people of India in the treatment of various

illness and diseases. In the literature, several authors have been reportedaboutthe antioxidant activity [1], digestive stimulation action [11],anti-toxin[73], anti-platelet aggregation [30], anti-inflammatory[27], anti-allergic35, virucidal31, anti-leukemic[12], anti-Severe Acute Respiratory Syndrome (SARS) and anti-cancer [36] properties of *H. cordata*. It has also been reported that *H. cordata* carries a range of antibacterial, anti-microbial, and immunomodulatory effects [30,13,27].

Previous studies revealed that *H. cordata* contain the biologically active compounds such as flavones, phenolic components (quercetin and chlorogenic acid), essential oil and alkaloids [5, 45, 50]. Prior reports on biochemical and epidemiologic results recommended that *H. cordata* contain phenolic and antioxidativecomponents[13] which responsible for free radical-scavenging activity that would be beneficial to the health of humans and animals [72, 42]. However, no information is available on the antioxidant activities of different parts of *H. cordata* to support practical application in the medicinal industry. Therefore, the present study was conducted to evaluate the antioxidant and genoprotective activities of three different (aqueous, ethanolic and methanolic) extracts from leaf and root of aromatic herb, *H.cordata*.

II. Material and Methods

2.1. Collection, identification and extraction process of plant sample

The whole plant,*Houttuyniacordata*(HC) were collected from Palove village,Pakke-Kessang (at elevation of 1100msl and the latitude of (27º14' N) and longitude (93º61' E) District East Kameng, Arunachal Pradesh, India; in the month of July-August, 2016 and it wasindividually washed with tap water. Further, leaf and root were carefully separated and spread under shade in the bamboo carpet. Once shadow dried, it was packed for transportation and brought to the Laboratory of Fish Nutrition and Feed Technology, ICAR-Central Institute of Fisheries Education (CIFE), Mumbai, India, for separation of crude extracts of leaf and root. The sample was deposited and voucher specimen number TT-33 and identified in Blatter Herbarium, St. Xavier's College, Mumbai, India.

2.2. Chemicals used for the extraction process

Agarose, bromocresol purple, D-glucose, Folin–Ciocalteu reagent, and methanol were purchased from Sigma Chemical Co. (St. Louis, MO,USA); Davis–Mingioli salt, deionised water, hydrochloric acid, hydrogen peroxide, phosphate buffer saline (PBS) and Triton-X 100 were procured from Sigma-Aldrich Co. (Steinheim, Germany); gallic acid and Tris-Acetate EDTA buffer were obtained from Merck (Darmstadt, Germany); while DNA plasmid (pJET1.2) from Fermantas and Na_2CO_3 purchased from Merck company. All chemicals were of HPLC grades.

2.3. Extraction from leaf and root of *H. cordata*

The extraction method was followedperstandard protocol [79] with slightly modificationfor both ethanolic and methanolic extraction.After complete drying, the leaf and root were ground to powder using mixer grinder. Either leaf or root of each *Houttuyniacordata* (10g) powder was taken into a 250ml flask and added with 100ml 90% ethanol (ethanol: water 90:10 v/v)or 90% methanol (methanol: water 90:10 v/v). The ethanol/ methanol powdered leaf or root were kept on orbital shaker at 160 rpm for 24hrs. After 24hrs, the extracts of leaf or root were filtered using muslin cloth to exclude the leaf and root powder residue. The extraction was done twotimes and the filtrates pulled together were centrifuged at 10,000rpm at 4ºC for 5min and the supernatant was collected. The volume of supernatant was reduced by rotatory evaporator at temperature of 40-65ºC, rotor speed of 40rpm. A greasy material (crude ethanolic/methanolic extract) obtained was transferred to screwed-cap bottles, labelled and stored under refrigerated (4ºC) condition until use. Aliquots of known weight fromeach extract were freeze-dried and lyophilized (-95^{0}C±4^{0}C and 0.100 to 0.200±111 mbars) for estimation of yield on dry weight basis.

2.4. Aqueous extraction

The aqueous extraction method was carried out per the standard procedure [79] with some modifications.A known quantity of 10g of air dried powdered of leaf or root powder was taken in 250ml flask and added with 100ml of distilled waterfollowed by heating at temperature of 60ºC on magnetic stirrer cum hotplate (SPINOT, Tarson, India) for 2 hrs. It was filtered using muslin cloth to exclude the leaf and root powder residue. The extraction was done two times and the filtrates pulled together were centrifuged at 10,000rpm for 5min and the supernatants were collected. Then the supernatant was concentrated using lyophilisation at temperature of -95^{0}C ±4^{0}C and pressure of 0.100 to 0.200±111 mbars. A greasy material (crude water extract) obtained was transferred to screwed-cap bottles, labelled and stored under refrigerated (4ºC) condition until use.

2.5. Determination of the Extraction Yield

The extraction yield is a measure of the solvent efficiency to extract specific components from the original material. In the case of HC, it will give an idea about the extractability of phenolics under different solvents. It could be calculated per the standard method of Liu et al. (2008) [41] as follow formula:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the freeze-dried extract}}{\text{Weight of the original sample}} \times 100$$

2.5.2 Determination of Antioxidant Activity

2.5.2. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The radical scavenging activity of the extracts of leaf or root was tested against 2,2-Diphenyl-1-picrylhydrazyl (Sigma-Aldrich) radical following the method described by previous authors [10]with slight modification. Each extract sample's stock solution (0.5 g ml^{-1}) was diluted to final concentrations of (0.001, 0.0025, 0.005, 0.025, and 0.05mg ml^{-1}) in water, ethanol and methanol. Ten microliter (10µl) of crude herb extracts was placed in 3 test tubes and 2ml of 0.01M DPPH solution in methanol was added. The test tubes were incubated in dark for 30min, and the UV absorbance was read at 517nm. A blank solution containing the same amounted of methanol and DPPH was prepared and measured. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. All the measurements were taken in triplicates and the mean value was calculated using the following formula:

DPPH Scavenging Effect (%) = [(AB - AA) ×100/AB]; where AB is the absorption of blank sample and AA is absorption of tested crude herbal extract solution.

2.5.3. Estimation of total phenolic contents (TPC)

Total phenolic contents in the root or leaf extracts were determined per the method described by previous workers [43] with slight modification. Concentrations of 0.001, 0.0025, 0.005, 0.025, and 0.05mg ml^{-1} of herbal extracts were prepared from the stock solution. Thirty microlitre of each part herbal extract was taken in a test tube and made up to 3ml with distilled water.After 3min, 2ml of 20% sodium carbonate (Na_2CO_3) was added and mixed well. A blue coloured reaction was developed in each tube. The tubes were then placed in boiling water for 1min, cooled and absorbance was measured in spectrophotometer at 650nm against a reagent blank. The standard curve was prepared using gallic acid in methanol and linear dose-response regression curve was generated at absorbance of 650nm. The total phenoliccontents in the samples were calculated from the standard curve and the results were expressed as gallic acid equivalent per 100g dry weight of the mgGAE/100 g extract. All the measurements were taken in triplicates to calculate the mean value.

2.5.4. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out per the standard procedure [6]with some modifications. Diluted sample of 0.001, 0.025, 0.0125, 0.0025, and 0.05mg $ml^{-1}$of herb extracts were prepared using the two solvents. The FRAP reagent was prepared fresh daily and warmed to 37ºC in the water-bath prior to use. To 20µl of sample extracts, 1.8ml of the FRAP reagent, containing 25ml of 300mM acetate buffer pH 3.6 2.5ml of 10mM 2,4,6-tripyridyls-triazine (TPTZ) in 40mMHCl and 2.5ml of 20ml ferric chloride ($FeCl_3 \bullet 6H_2O$) solution was added, and the absorbance of the reaction mixture was read at 593nm after 4min. The standard curve was constructed using iron (II) sulfate solution (100-2000 µM), and the results were expressed as µM Fe (II)/g dry weight of herb material. All the measurements were taken in triplicate to calculate the mean value using the following formula:

FRAP value of sample (µM) = [(Δabs of sample from 0 to 4 min) / (Δabs of standard from 0 to 4 min) × FRAP value of standard (×2) (10^3 µM)].

2.5.5. Superoxide Anion Scavenging Activity (NTZ)

Measurement of superoxide anion scavenging activity of *Houttuyniacordata*root or leaf extract was based on the method described by previous workers [40]. Superoxide radicals were generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitrobluetetrazolium (NBT). In this experiment, the superoxide radicals were generated in 3ml of Tris-HCl buffer (16mM, pH 8.0) containing 1ml of NBT 50µM) solution, 1ml NADH (78µM) solution and sample solution of *Houttuyniacordata* ethanol extract 100 µg ml^{-1}) in water. The reaction started by adding 1ml of phenazinemethosulphate (PMS) solution (10µM) to the mixture. The reaction mixture was incubated at 25ºC for 5 min, and the absorbance was measured at 560nm in spectrophotometer against blank samples. L-ascorbic acid was used as a control. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:
Inhibition (%) = {[(A0-A1) ×100)]/A0}; where, A0 was the absorbance of the control, and A1 was the absorbance of *Houttuyniacordata*ethanolicextract sample and standard (L-ascorbic acid).

2.5.6. Haemolytic assay

Haemolytic activity was evaluated by using the standard method [53]. Fresh heparinised human blood (3ml) was gently mixed and dispensed into a sterile falcon tube (15 ml) and centrifuged for 5 min at 900 g. The sticky pellet was separated from the supernatant and washed with sterile isotonic phosphate buffer saline (PBS 2% v/v) solution thrice, to maintain the pH to ~7.4. The well shaken/pounded cells were added to chilled, sterile PBS buffer, as to get 7.068 × 108 cells ml^{-1}. With measured/optimised of different solvent and parts (namely leaf and root) extracts (20 µl) were mixed separately with diluted blood cell suspension (180µl) in separate microfuge tubes; each tube was incubated at 37°C for 35 min with continuous agitation (180 rpm). After incubation, supernatant (100µl) from each tube was mixed with 900 µl cooled, sterile PBS in separate microfuge tubes and retained on ice (5 min). Absorbance was then measured at 576 nm, using Triton-X 100 (0.1%) as the positive control. From the absorbance of the sample and the positive control, haemolytic activity was quantified using the following formula:

Haemolysis (%) = [absorbance of sample / absorbance of standard] ×100; where, standard indicates positive control, which show 100 % haemolysis.

2.5.7. Genoprotective assay

Genoprotective potential of samples was assessed on DNA plasmid (pJET1.2)[66] with sight modification. In this assay, the protection capability of the extracts against oxidative damage to DNA instigated by H_2O_2 and UV radiations was measured. The trials were conducted in microcentrifuge tubes containing pJET1.2 DNA plasmid (3L, 172 ngL^{-1}), herbal extract (5µL, in varying concentrations of 25 mg ml^{-1}) and H_2O_2(2% µl of 30%). A negative control (devoid of sample) and a positive control (without H_2O_2) were also run along with the samples. All eppendorf tubes except the positive controls were also exposed to UV radiations for 15 min to break down the supercoiled DNA plasmid. After completion of reaction, the reaction mixtures were loaded on 1% agarose gel along with loading dye (6×) using Tris Acetate–EDTA buffer (1×). The gel was photographed using gel documentation system (Gene Genius, SYNGENE) applying software Syngene (version 4.01.00) after staining with ethidium bromide (0.5µg ml^{-1}).

2.7. Statistical analysis

The data were statistically analysed by statistical package SPSS version 16. Comparisons of different assays of the extracts were done byone way ANOVA and the comparison among different mean values were made using Duncan's Multiple Range Test (Duncan, 1955). All the comparisons were made at the 5% ($P<0.05$) probability levels.

III. Results and Discussion

3.1. Yield

Figure 1 represents the percentage of extract yield for three HCparts with different extracting solvents and yield result were found to be 7.67^{a}±0.33 to 9.33^{bc}±0.58 % of root to leaf in aqueous solvent, 9.00^{cd}±0.58 to 10.67^{ab}±0.33% of root to leaf extract of ethanolic solvent and 11.33^{d}±0.33 to 13.67^{e}±0.58 % of root to leaf methanolic solventon dry weight basis of aqueous to methanolic solvent extractions which were within the range19.28gof previous report[80]. Methanolicwas found superior for producing better yield of extract as compared to ethanolic and aqueous solvents. Improved efficiency of methanolic than ethanolic and aqueous extract can be linked with its ability to break down the cellular structure; hence the liberation of bound phytoconstituents of a plant matrix. Different factors like temperature, pH, and solvent polarities, solvent to sample ratio and chemical nature of sample may affect the recovery of phytochemicals from plant which was agreement with previous report [76].

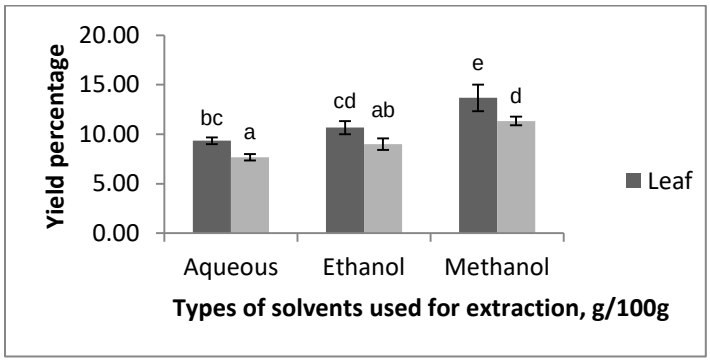

Figure 1. Yield percentage of leaf and root HC extracts using different solvents

Data expressed as mean±SE (n=3). Mean values in the same column with different superscript differ significantly (P<0.05).

3.2. Radical scavenging method - 2, 2 -Diphenyl-1-pcrylhydrazyl (DPPH)

DPPH assay can be used in the herbal/ plant components to determine their ability of antioxidant activities to scavenge the free radicals [9, 54]. Free radicals are known for their biological damages to the cells through oxidative stress process. DPPH assay is a most preferable method for the herbal samples because DPPH is a synthetic, stable radical that does not degenerate in water, methanol, or ethanol [2]. The principle of DPPH method is relies on the reduction of DPPH [29]as DPPH easily collects the electron or hydrogen from antioxidant molecules to turn into a stable molecule [61]. Consequently, the antioxidant activity of DPPH is measured based on the ability of antioxidant molecules present in the extract to donate hydrogen to the free radical scavengers to make them as a stable molecules [22]. Free radical scavenging activity is measured by the discoloration process because when DPPH radicals kept in a methanol solution along with an antioxidant agent, DPPH will be converted into DPPH-H (diphenylhydrazine) molecules, during this process colour changes, which reflects to be a radical scavenging activity of the any drug analysed [44, 26]. More the colour changes, more the free radical scavenging activity as it is directly related to the number of electrons captured [23,29]. This method is acceptable for analysing any drug which claimed to have antioxidant potential [68]. In the present study, the radical scavenging capacity of the *H. cordata*leaf and root extracts with different solvents were tested using stable free radical, DPPH and the values are represented as inhibition (%). The radical scavenging activity exhibited by aqueous, ethanolic and methanolic HC extracts at a concentration of 0.025mgml^{-1}was shown (Table 1). The leaf extract exhibited significantly higher (P<0.05) DPPH activity, than the root extract. While comparing solvents, the methanolic and ethanolic extracts showed significantly (P<0.05) higher DPPH activity than aqueous extracts . The activity of different HC extracts are found to be in thedescending order from highest to lowest: ML>EL>AL>ER>MR>AR for DPPH. The highest DPPH scavenging effect was found in the methanolic extract of leaf (75.30±2.16) and the least effect was noticed in the ethanolic extract of root (28.69±1.03). The results from the present study showed the leaf extract possess highest radical scavenging activity than the root extract irrespective of the solvent used and organic solvents performed better than aqueous extracts in terms of DPPH activity.

3.3. Total phenolic content assay (TPC)

Plant/herbal extract contain significant amounts of total phenolic contents [74], which is directly correlated with the degrees of anti-oxidative capacity [11, 57,39]. It is also reported that phenolic compounds from the plants are responsible for providing the defence against the reactive oxygen species (ROS) during its damage to cells [55, 74, 56]. It was reported that phenolic compounds extracted from herbs possess excellent free-radical-scavenging activity [34,55] thus can be used for the commercial applications [69]. The leaf extract has shown higher total phenolic content than root extracts irrespective of the solvent used (Table. 1). Among different solvents, ethanol and methanol were more efficient in extracting total phenolic content than water for both leaf and root extract. The activity of different HC extracts isfound to be in the descending order from highest to lowest: ML>EL>AL>ER>MR>AR for TPC (table. 1). The highest TPC values found in the methanolic HC leaf extract(305.68±1.58) and lowest in aqueous HC root extract (66.68±1.58) at the given concentration (0.025mgml^{-1}). The values obtained for the TPC in the present study lies within the range (0.75 to 12.4 mg/g) as described by previous authors using the HC extract [11].Like DPPH activity, the leaf extract showed highest TPC content than the root extract counterpart irrespective of any solvent used. Among the solvents, organic solvents (Ethanol and methanol) could extract more phenolic compounds as compared to water.

3.4. Ferric reducing antioxidant power assay (FRAP)

FRAP method is used to determine the antioxidant capacity of the herbal extracts based on the principle that FRAP reagent contains TPTZ- Fe (III) complex, which is reduced to TPTZ- Fe (II) complex as a result of free radical scavenging by donating an electrons to reactive radicals in order to convert them into stable and unreactive species [70,69]. It is believed that ability of reducing TPTZ- Fe (III) to TPTZ- Fe (II) may be due to total phenolic contents and its antioxidant potential [71, 74]. The leaf

extracts were having higher FRAP activity than root extracts irrespective of the solvent used. Methanolic extracts has shown significantly higher ($P<0.05$) FRAP activity than aqueous and ethanolic extracts. The activity of different HC extracts are concentration dependent which are given in the descending order from highest to lowest: ML>EL>AL>ER>MR>AR for FRAP (Table. 1). In the present study, FRAP assay for different HC extracts was done which exhibited the highest value for ethanolic extract (20.49±0.58) and lowest in aqueous root extract (5.17±0.13) at the given concentration ($0.025 mgml^{-1}$) given (Table 1).Results demonstrated that total antioxidant capacity measured by the FRAP method between the different extracts of HC showed good antioxidant potential. The reason may be involved with the presence of higher amounts of total phenolic contents and other antioxidant molecules in HC as supported by earlier reports[71, 74] in different herbal extracts. Overall results indicated leaf extract showed higher FRAP activity than the root extract counterparts irrespective of the solvent used.

3.5. Superoxide anion radical scavenging assay (NTZ)

Superoxide anion radical scavenging assay (NTZ) is mainly used to identify the antioxidative potential of the any drugs [67, 52]. Superoxide anion radicals are dangerous to the cells and they generate more reactive radicals[49]. During the oxidation condition of biomolecules, the reactive molecules of superoxide anion radical (O2•-) are generated with the four-electron reduction of molecular oxygen into water. Superoxide anion radicals (O2•-) generated from the cells produce highly reactive oxygen derived radicals which are responsible for the oxidative damage of biomolecules. Oxidative damagecauses many pathological disorders and diseases [28]. Antioxidant molecules help in protecting the cells from the oxidative damages [37]. However, when oxidative potential cross beyond the antioxidant capacity of cells, It would result in a variety of diseases [25]. It is reported that many herbs/spices have excellent antioxidant properties [42, 25]. However, there is no information about *in vitro* superoxide anion activity of HC. Root extracts showed better NTZ activity ($P<0.05$) compared to leaf extracts while among the three solvents, water was bestin NTZ activity. The activity of different HC extracts are concentration dependent which are given in the descending order from highest to lowest: AR>ER>AL>MR>EL>ML for NTZ (table.1). Unlike other assays, NTZ activity was higher for the root extract than the leaf extract.In the results, NTZ activity was highest in aqueous root extract (92.92±0.90) and lowest in methanolic leaf extract of HC (80.96±0.80) at $0.025 mgml^{-1}$which is in agreement with previous reports [78]. The results from this study provided clear evidence that root extracts of HC have superoxide anion scavenging activity and aqueous extracts were shown better NTZ activity. Our results aresupported by previous findings in the literature using different herbal extracts [42,25, 43].

3.6. Haemolytic assay

In vitro haemolysis test for human erythrocytes is generally used as an index to determine the free radical-induced damage in the biological membranes [63]. Medicinal aromatic plant tissues may possess secondary metabolites that prove to be destructive to human blood cells. These metabolites damage the cell membranes of human being erythrocytes by counteracting with different lipid compositions present in the cell membranes. The lower the haemolytic activity in a plant extract the greater the support for its application in medicinal formulations, while greater haemolytic effects give an indication of cytotoxicity of plant extracts. Thus, haemolytic assays are necessary to confirm whether a plant extract having high antioxidants and biological activities that could be used in medicinal and feed, formulations [32, 38]. In this study, the cytotoxicity of different leaf and root extracts of HC was evaluated by an *in vitro* haemolytic assay (Table 1).The activity of different HC extracts are follows the order from highest to lowest: AL>AR>ML>EL>MR>ER for HA(%)(Table 1). Results showed that aqueous extract has shown higher haemolysis/cytotoxicity effect than ethanolic and methanolic HC extracts of both root and leaf at 0.25 mg ml^{-1} concentration. The present results of highest haemolytic effect in the aqueous extracts can be explained that non polar solvents may hydrolyse the haemolysis or cytotoxic capacity of herbal extract and whereas polar solvents like aqueous extract keep intact the potentiality of haemolysis or cytotoxic capacity [76]. These results are in line with the earlier reports using the extract of Amaranthaceaeplants [76]. It is also reported that inhibition percentage of haemolysis of red blood cells is directly related to the total phenolic contents [14,4] as well as flavonoids and their glycosides [8, 63, 64]. In the present study, the haemolytic activity against human erythrocytes may be also because of the presence of phenolic compounds as well as flavonoids and their glycosides in *H. cordata* extracts are effective against the toxic metabolites like alkaloids and saponins, which may break cell membranes of erythrocytes [31, 32, 77].

Table 1. Antioxidants and biological activities of different leaf and root HC extracts at the concentration of 0.025g/ml

Type of extract	DPPH (Inhibition %)	TPC (mgGAE/100 g dry weight of extract)	FRAP (µmolFe(II) /g dry weight of extract)	NTZ (Inhibition %)	HA (%)
Leaf	64.36^{b}±1.72	231.53^{b}±2.49	16.37^{b}±0.22	84.23^{a}±0.28	49.67^{b}±1.70
Root	34.70^{a}±1.72	97.20^{a}±2.49	6.98^{a}±0.22	90.29^{b}±0.2	36.68^{a}±1.70
Type of solvent					
A	37.57^{a}±2.11	96.96^{a}±3.04	8.95^{a}±0.27	90.62^{c}±0.35	58.69^{b}±2.08
E	57.70^{b}±2.11	186.84^{b}±3.04	10.75^{b}±0.27	86.54^{b}±0.3	33.43^{a}±2.08
M	53.33^{b}±2.11	209.30^{b}±3.04	15.34^{c}±0.27	84.62^{a}±0.35	37.41^{a}±2.08
Simple main effects					
AL	46.44^{c}±1.05	127.24^{c}±10.48	12.72^{c}±0.47	88.31^{c}±0.31	65.62^{e}±0.58
AR	28.69^{a}±1.03	66.68^{a}±0.43	5.17^{a}±0.13	92.92^{d}±0.90	51.76^{d}±0.44
EL	71.35^{d}±2.37	261.68^{d}±0.58	15.90^{d}±0.79	83.40^{b}±0.40	39.08^{c}±0.18

ER	44.05^{bc}±1.07	112.01^{b}±0.65	5.60^{a}±0.04	89.67^{c}±0.96	27.77^{a}±0.18
ML	75.30^{d}±2.16	305.68^{e}±0.58	20.49^{d}±0.01	80.96^{a}±0.80	51.68^{d}±0.25
MR	39.57^{b}±2.52	112.93^{b}± 0.43	10.18^{b}±0.12	88.27^{c}±0.27	30.51^{b}±0.3
Part	**S**	**S**	**S**	**S**	**S**
Solvent	**S**	**S**	**S**	**S**	**S**
Part × solvent	**S**	**S**	**S**	**NS**	**NS**

Data expressed as Mean±SE. Different superscript (a, b, c,) in the same column under each category signify statistical differences (S indicates P<0.05, NS indicates P>0.05).

DPPH-2, 2-Diphenyl-1-picrylhydrazyl assay; TPC-Total phenolic contents; FRAP- Ferric reducing antioxidant power assay; NTZ-Superoxide anion radical scavenging assay; HA-Haemolytic assay; GA-Genoprotective assay
AL-Aqueous leaf extract, AR-Aqueous root extract, EL-Ethanolic leaf extract, ER- Ethanolic root extract, ML-Methanolic leaf extract, MR-Methanolic root extract.

3.7. Genoprotective activity

Genoprotective activity assay is generally adopted for herbal/plant extracts to determine their ability to protect them against oxidative damage to cellular DNA [60, 44]. This method is reliable because under the ultraviolet radiation, H_2O_2 generate HO, which will break the DNA backbone (nitrogenous bases), open the supercoiled arrangement of DNA, consequently damages the DNA [59]. In the present study, the aqueous, ethanolic and methanolic extracts of HC from two different parts were also tested for their genoprotective effects with the same concentration levels (0.25 mg ml^{-1}) used for antioxidant and haemolyic assays. Figure 2 represents electropherograms for the genoprotective activity assay of leaf and root HC extracts; also figure 3 shows the % genoprotection when compared with positive control for the different types of extracts evaluated. In general, significantly highest (P<0.05) genoprotective activity was recorded for the root extract than their leaf extract counterpart. Among the different extraction methods, aqueous extraction was found best (P<0.05) in terms of genoprotetive activity. The activity of different HC extracts follows the order from highest to lowest: AR>MR>ER>EL>AL>ML for GA (%).Genoprotective activity was higher in the AR followed by MR and significantly lower (P<0.05)genoprotection was noticed in ML which was significantly lesser than all other extracts. Results demonstrated that HC extracts from two different parts of HC (lanes AL, AR, EL, ER, ML, MR) showed a large degree of protection against DNA damage which is comparable in figure 2 based on the presence and intensity of bands with standard positive control (lane +VE), while negative control (lane -VE) without HC extracts were found to be not effective (no bands). The genoprotective effect of HC observed in the present study can be explained due to the presence of high phenolic contents or flavonoids in the HC extracts perhaps protected the DNA plasmid against cytotoxic and genotoxic activities as reported by [33,47] using other herbal extracts.

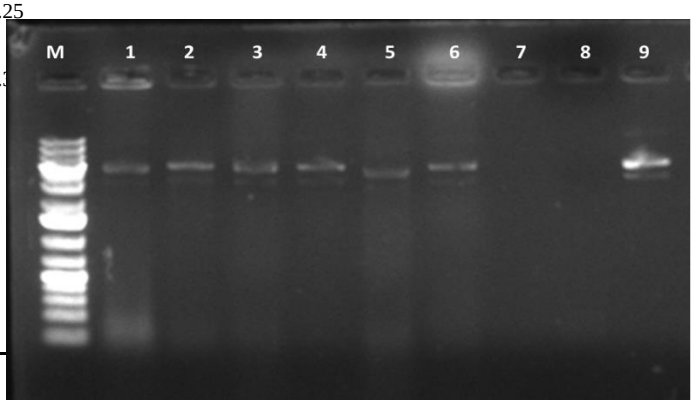

Figure 2. Percentage of genoprotection of different leaf and root HC extracts

Notation: M-1KD, Reference ladder, 1- Aqueous leaf extract, 2-Aqueous root extract, 3-Ethanolic leaf extract, 4- Ethanolic root extract, 5-Methanolic leaf extract, 6-Methanolic root extract, 7-Blank, 8-Negative control, 9-Positive control.

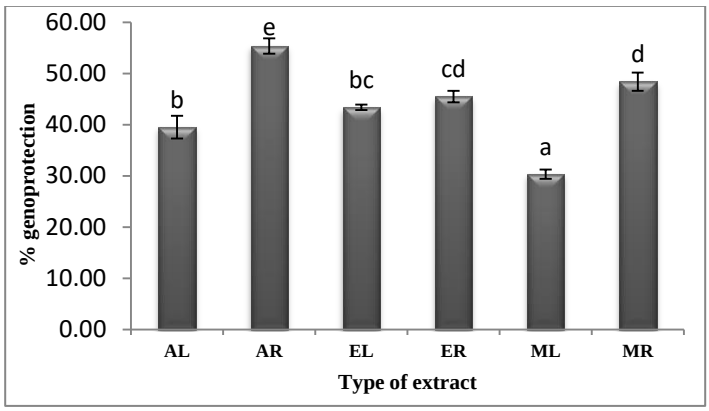

Figure 3. Percentage of genoprotection of different leaf and root HC extracts

Data expressed as mean±SE (n=3). Mean values in the same column with different superscript differ significantly (P<0.05).
AL-Aqueous leaf extract, AR-Aqueous root extract, EL-Ethanolic leaf extract, ER- Ethanolic root extract, ML-Methanolic leaf extract, MR-Methanolic root extract.

IV. Conclusion

The evaluation of antioxidant and biological assay performed with aqueous, ethanolic and methanolic extraction revealed that leaf and root of *Houttuyniacordata* is endowed with potentially exploitable antioxidants that is free radical scavenging and biological active compounds. However, the overall results is indicated that leaf extract is superior to root extractin both antioxidative and biological properties except for NTZ and Haemolytic activities assay which ethanolic and methanolic extract showed low activities.However, the parameters indicating antioxidative property such as total phenolic content, DPPH, scavenging capacity and ferric reducing antioxidant power has found to be higher for ethanolic and

methanolic extract compared to aqueous extracts.Maenwhile, the biological activities such as haemolytic and genoprotective activities were higher in aqueous extracts than that of methanolic and ethanolic extracts. Results from the present study demonstrated that HC possess significantly good amounts of total phenolic content that can be used as natural antioxidants for the commercial applications in the terrestrial and aquatic animal feeds to replace the synthetic antioxidants such as butylated hydroxyl toluene (BHT) and butylatedhydroxyl anisole (BHA) which are criticised in the feeds as supported by earlier reports[69].Three different types of extracts aqueous ethanolic and methanolic were established to possess highest superoxide anion assay, haemolytic and genoprotection activities assays. Different solvents have different capacity to extract antioxidative compounds and biologically active compounds.Despite these differences, all the HC extracts using aqueous, ethanolic and methanolicsolvents showed considerably higher antioxidant and biological effects; therefore, HC extracts could be utilized as a natural source of antioxidants for human, terrestrial and aquatic animals. Nevertheless, it is suggested that further works may be done with *in vivo* test in terrestrial and aquatic animals to support HC extracts as an excellent antioxidants source for the application in the pharmaceutical industries.

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REFERENCES

[1] Aaby,K., Hvattum, E. and Skrede, G., Analysis of flavonoids and other phenolic compounds using high-performance liquid chromatography with coulometric array detection: relationship to antioxidant activity. Journal of agricultural and food chemistry : 2004: 52(15), pp4595-4603.

[2] Aksoy, L., Kolay, E., Ağılönü, Y., Aslan, Z. and Kargıoğlu, M., Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic *Thermopsisturcica*. Saudi journal of biological sciences, 20(3), 235-239 (2013).

[3] Amaeze, O.U., Ayoola, G.A., Sofidiya, M.O., Adepoju-Bello, A.A., Adegoke, A.O. and Coker, H.A.B., Evaluation of antioxidant activity of *Tetracarpidiumconophorum* (Müll. Arg) Hutch &Dalziel leaves. Oxidative medicine and cellular longevity. 2011: 7, pp701-976.

[4] Barros, L., Ferreira, M.J., Queiros, B., Ferreira, I.C. and Baptista, P., Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food chemistry. 2007:103(2), pp413-419.

[5] Bauer, R., Proebstle, A., Lotter, H., Wagner-Redecker, W. and Matthiesen, U., Cyclooxygenase inhibitory constituents from *Houttuyniacordata*. Phytomedicine, 1996: 2(4), pp305-308.

[6] Benzie, I.F. and Strain, J.J., The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry, 1996: 239(1), pp70-76.

[7] Bhattacharyya, N. and Sarma, S., Assessment of availability, ecological feature, and habitat preference of the medicinal herb *Houttuyniacordata*Thunb in the Brahmaputra Valley of Assam, India. Environmental monitoring and assessment, 2010: 160(1-4), pp277.

[8] Blasa, M., Candiracci, M., Accorsi, A., Piacentini, M.P. and Piatti, E., Honey flavonoids as protection agents against oxidative damage to human red blood cells. Food Chemistry, 2007: 104(4), pp.1635-1640.

[9] Bozin, B., Mimica-Dukic, N., Bogavac, M., Suvajdzic, L., Simin, N., Samojlik, I. and Couladis, M., Chemical composition, antioxidant and antibacterial properties of *Achilleacollina* Becker ex HeimerlSl and *A. pannonica* Scheele essential oils. Molecules, 2008: 13(9), pp. 2058-2068.

[10] Brand-Williams, W., Cuvelier, M.E. and Berset, C.L.W.T., Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology, 1995: 28(1), pp. 25-30.

[11] Cai, Y., Luo, Q., Sun, M. and Corke, H., Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life sciences*, 2004: 74(17), pp. 2157-2184.

[12] Chang, J.S., Chiang, L.C., Chen, C.C., Liu, L.T., Wang, K.C. and Lin, C.C., Antileukemic activity of *Bidenspilosa* L. var. minor (Blume) Sherff and *Houttuyniacordata*Thunb. The American journal of Chinese medicine, 2001: **29**(02), pp. 303-312.

[13] Chen, Y.Y., Liu, J.F., Chen, C.M., Chao, P.Y. and Chang, T.J., A Study of the Antioxidative and Antimutagenic Effects of HouttuyniacordataThunb. Using an Oxidized Frying Oil-Fed Model, Journal of Nutritional Science and Vitaminology, 2003: 49(5);pp. 327-333.

[14] Cheung, L.M., Cheung, P.C. and Ooi, V.E., Antioxidant activity and total phenolics of edible mushroom extracts. Food Chemistry, 2003: 81(2), pp. 249-255.

[15] Dekkers, J.C., van Doornen, L.J. and Kemper, H.C., The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage. Sports Medicine, 1996: 21(3), pp. 213-238.

[16] Deshpande, S.S., Madhavi, D.L. and Salunkhe, D.K. eds., Food Antioxidants: Technological, Toxicological, and Health Perspectives, (1996).

[17] Dinis, T.C., Almeida, L.M. and Madeira, V.M.C., Lipid peroxidation in sarcoplasmic reticulum membranes: effect on functional and biophysical properties. Archives of biochemistry and biophysics, 1993: 301(2), pp. 256-264.

[18] Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P. and Vidal, N., Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food chemistry, 2006: 97(4), pp. 654-660.

[19] Dragland, S., Senoo, H., Wake, K., Holte, K. and Blomhoff, R., Several culinary and medicinal herbs are important sources of dietary antioxidants. The Journal of nutrition, 2003: 133(5) pp. 1286-1290.

[20] Dražar, P. and Moravcova, J., Recent advances in analysis of Chinese medical plants and traditional medicines. Journal of Chromatography B, 2004: 812(1) pp. 3-21.

[21] Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D. and Guo, Z., Medicinal Plants in Therapy. Bulletin of the World Health Organization, 1985: 63(6), pp. 965-981.

[22] Fukumoto, L.R. and Mazza, G., Assessing antioxidant and prooxidant activities of phenolic compounds. Journal of agricultural and food chemistry, 2000: 48(8), pp. 3597-3604.

[23] Ghosh, M.N, Fundamentals of Experimental Pharmacology, 2nd Edn., Scientific Book Agency, Calcutta, 1998: pp. 174-179.

[24] Grillo, C.A. and Dulout, F.N., Cytogenetic evaluation of butylatedhydroxytoluene. Mutation Research/Genetic Toxicology, 1995: 345(1-2), pp.73-78.

[25] Gülçin, İ., Oktay, M., Küfrevioğlu, Ö.İ. and Aslan, A., Determination of antioxidant activity of lichen Cetrariaislandica (L) Ach. Journal of ethnopharmacology, 2002: 79(3), pp. 325-329.

[26] Guo, Y.H. and Xu, J.Y., Extraction and purification of *Houttuyniacordata* flavonoids and identification of flavonoids type. Food Science, 2007: 9,pp.070.

[27] Lu, H., Wu, X., Liang, Y. and Zhang, J., "Variation in Chemical Composition and Antibacterial Activities of Essential Oils from Two Species of HouttuyniacordataThunb," Chemical & Pharmaceutical Bulletin, 2006: 54(7), pp. 936-940.

[28] Halliwell, B., Gutteridge, J.M.C., Free radicals in biology and medicine. Oxford, UK: Oxford University Press: (1985).

[29] Harborne, A.J., Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media (1998).

[30] Hayashi, K., Kamiya, M. and Hayashi, T., Virucidal effects of the steam distillate from *Houttuyniacordata*and its components on HSV-1, influenza virus, and HIV. Plantamedica, 1995: 61(3), pp. 237-241.

[31] Hayashi, K., Kamiya, M. and Hayashi, T., Virucidal Effects of the Steam Distillate from *Houttuyniacordata* and Its Components on HSV-1, Influenza Virus, and HIV. PlantaMedica, 2005: 61(3), pp. 237-241.

[32] Inalegwu, B. and Sodipo, O., Phytochemical screening and haemolytic activities of crude and purified saponins of aqueous and methanolic extracts of leaves of *Tephrosiavogelii* Hook. F. Asian J Plant Sci. Res, 2013: 3(5), pp. 7-11.

[33] Jaiswal, S., Mansa, N., Prasad, M.P., Jena, B.S. and Negi, P.S., Antibacterial and antimutagenic activities of *Dilleniaindica* extracts. Food Bioscience, 2014: 5, pp. 47-51.

[34] Khan, A.W., Jan, S., Parveen, S., Khan, R.A., Saeed, A., Tanveer, A.J. and Shad, A.A., Phytochemical analysis and enzyme inhibition assay of *Aervajavanica* for ulcer. Chemistry Central Journal, 2012: 6(1), pp. 76.

[35] Kim, D.O. and Lee, C.Y., Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolic in scavenging a free radical and its structural relationship. Critical reviews in food science and nutrition, 2004: 44(4), pp. 253-273.

[36] Kim, G.S., Kim, D.H., Lim, J.J., Lee, J.J., Han, D.Y., Lee, W.M., Jung, W.C., Min, W.G., Won, C.G., Rhee, M.H. and Lee, H.J., Biological and antibacterial activities of the natural herb *Houttuyniacordata* water extract against the intracellular bacterial pathogen salmonella within the RAW 264.7 macrophage. Biological and Pharmaceutical Bulletin, 2008: 31(11), pp.2012-2017.

[37] Kim, S.K., Ryu, S.Y., No, J., Choi, S.U. and Kim, Y.S., Cytotoxic alkaloids from *Houttuyniacordata*. Archives of pharmacal research, 2001: 24(6) 518-521.

[38] Kourounakis, A.P., Galanakis, D., Tsiakitzis, K., Rekka, E.A. and Kourounakis, P.N., Synthesis and pharmacological evaluation of novel derivatives of anti-inflammatory drugs with increased antioxidant and anti-inflammatory activities. Drug Development Research, 1999: 47(1), pp. 9-16.

[39] Lamien-Meda, A., Kiendrebeogo, M., Compaoré, M., Meda, R.N., Bacher, M., Koenig, K., Pacher, T., Fuehrer, H.P., Noedl, H., Willcox, M. and Novak, J., Quality assessment and antiplasmodial activity of West African *Cochlospermum* species. Phytochemistry, 2015: 119**,**pp. 51-61.

[40] Liu, F., Ooi, V.E.C. and Chang, S.T., Free radical scavenging activities of mushroom polysaccharide extracts. Life sciences, 1997: **60**(10), pp. 763-771.

[41] Liu, H., Qiu, N., Ding, H. and Yao, R., Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. Food Research International, 2008: 41(4), pp. 363-370.

[42] Lu, H.M., Liang, Y.Z., Wu, X.J. and Qiu, P., Tentative fingerprint-efficacy study of *Houttuyniacordata* injection in quality control of traditional Chinese medicine. Chemical and pharmaceutical bulletin, 2006: 54(5), pp. 725-730.

[43] Madsen, H.L. and Bertelsen, G., Spices as antioxidants. Trends in Food Science & Technology, 1995: **6**(8), pp.271-277.

[44] Malik, C.P. and Singh, M.B., Plant enzymology and histo-enzymology, (1st Edn.).Kalyani Publishers, New Delhi, 1980: pp. 286-288.

[45] Mayne, S.T., Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. *The Journal of nutrition*, 2003: 133(3) pp. 933S-940S.

[46] Meng, J., Leung, K.S.Y., Jiang, Z., Dong, X., Zhao, Z. and Xu, L.J., Establishment of HPLC-DAD-MS fingerprint of fresh *Houttuyniacordata*. Chemical and pharmaceutical bulletin, 2005: 53(12), pp. 1604-1609.

[47] Molyneux, P., The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci. Technol, 2004: 26(2), pp. 211-219.

[48] Mufti, F.U.D., Ullah, H., Bangash, A., Khan, N., Hussain, S., Ullah, F., Jamil, M. and Jabeen, M., Antimicrobial activities of *Aervajavanica* and *Paeoniaemodi* plants. Pak. J. Pharm. Sci, 2012: 25(3), pp. 565-569.

[49] Ng, L.T., Yen, F.L., Liao, C.W. and Lin, C.C., Protective effect of Houttuyniacordata extract on bleomycin-induced pulmonary fibrosis in rats. The American journal of Chinese medicine, 2007: 35(3), pp. 465-475.

[50] Nishikimi, M., Rao, N.A. and Yagi, K., The occurrence of superoxide anion in the reaction of reduced phenazinemethosulfate and molecular oxygen. Biochemical and biophysical research communications, 2012: 46(2), pp. 849-854.

[51] Patra, J.K., Rath, S.K., Jena, K., Rathod, V.K. and Thatoi, H., 2008. Evaluation of antioxidant and antimicrobial activity of seaweed (*Sarqassum* sp.) extract: A study on inhibition of Glutathione-S-Transferase activity. Turkish Journal of Biology, 2008: 32(2), pp. 119-125.

[52] Pavrez, M., Mahboob, H.K., Zahuul, I., Shek, M.H., Antimicrobial activities of the petroleum ether, methanol and acetone extracts of kaempferia galangal. rhizome.J. Life Earth Sci., 2005: 1, pp. 25–29.

[53] Peschel, W., Sánchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzía, I., Jiménez, D., Lamuela-Raventos, R., Buxaderas, S. and Codina, C., An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chemistry, 2006: 97(1), pp. 137-150.

[54] Powell, W.A., Catranis, C.M. and Maynard, C.A., Design of self-processing antimicrobial peptides for plant protection. Letters in applied microbiology, 2000: 31(2),pp. 163-168.

[55] Pyrzynska, K. and Pękal, A., Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples. Analytical Methods, 2013: 5(17), pp.4288-4295.

[56] Rice-Evans, C.A., Miller, N.J. and Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free radical biology and medicine, 1996: 20(7), pp.933-956.

[57] Sengul, M., Yildiz, H., Gungor, N., Cetin, B., Eser, Z. and Ercisli, S., Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pak J Pharm Sci, 2009: 22(1),pp. 102-106.

[58] Shan, B., Cai, Y.Z., Sun, M. and Corke, H., Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. Journal of agricultural and food chemistry, 2005: 53(20), pp. 7749-7759.

[59] Sharma, N., Bhardwaj, R., Kumar, S. and Kaur, S., Evaluation of Bauhinia variegata L. bark fractions for in vitro antioxidant potential and protective effect against H2O2-induced oxidative damage to pBR322 DNA. African Journal of Pharmacy and Pharmacology, 2011: 5(12), pp. 1494-1500.

[60] Shanab, S.M., Antioxidant and antibiotic activities of some seaweeds (Egyptian isolates). Int J AgricBiol, 2007: 9(2), pp.220-225.

[61] Shi, Y.L., James, A.E., Benzie, I.F. and Buswell, J.A., Mushroom-derived preparations in the prevention of H_2O_2-induced oxidative damage to cellular DNA. Teratogenesis, carcinogenesis, and mutagenesis, 2002: 22(2), pp.103-111.

[62] Soare, J.R., Dinis, T.C., Cunha, A.P. and Almeida, L., Antioxidant activities of some extracts of *Thymus zygis*. Free radical research, 1997: 26(5), pp.469-478.

[63] Stephens, N.G., Parsons, A., Brown, M.J., Schofield, P.M., Kelly, F., Cheeseman, K. and Mitchinson, M., Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). The Lancet, 1996: 347(9004), pp.781-786.

[64] Tabart, J., Kevers, C., Pincemail, J., Defraigne, J.O. and Dommes, J., Comparative antioxidant capacities of phenolic compounds measured by various tests. Food Chemistry, 2009: 113(4), pp.1226-1233.

[65] Tepe, B., Degerli, S., Arslan, S., Malatyali, E. and Sarikurkcu, C., Determination of chemical profile, antioxidant, DNA damage protection and antiamoebic activities of Teucriumpolium and Stachysiberica. Fitoterapia, 2011: 82(2),pp. 237-246.

[66] Tailor, C.S. and Goyal, A., Antioxidant Activity by DPPH Radical Scavenging Method of *Ageratum conyzoides* Linn. Leaves, American Journal of Ethnomedicine, 2014: 1(4) pp. 244-249.

[67] Tsai, C.H., Chang, R.C., Chiou, J.F. and Liu, T.Z., Improved superoxide-generating system suitable for the assessment of the superoxide-scavenging ability of aqueous extracts of food constituents using ultraweakchemiluminescence. Journal of agricultural and food chemistry, 2003: 51(1) pp.58-62.

[68] Wagner, H. and Bladet, S., Plant Drug Analysis-A TLC Atlas, 1st Edn, Springer verlag Berlin, Heidel berg, New York, 1996: pp. 195-214.

[69] Wojdyło, A., Oszmiański, J. and Czemerys, R., Antioxidant activity and phenolic compounds in 32 selected herbs. Food chemistry, 2007: 105(3) pp.940-949.

[70] Wong, C.C., Li, H.B., Cheng, K.W. and Chen, F., A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food chemistry, 2006: 97(4), pp.705-711.

[71] Yıldırım, A., Mavi, A., Oktay, M., Kara, A.A., Algur, Ö.F. and Bilaloğlu, V., Comparison of antioxidant and antimicrobial activities of Tilia (*Tiliaargentea*Desf ex DC), sage (*Salvia triloba* L.), and Black tea

(*Camellia sinensis*) extracts. Journal of Agricultural and Food Chemistry, 2000: 48(10) pp. 5030-5034.

[72] Chen, Y.Y. Chen, C.M., Chao, P.Y., Chang, T.J. and Liu,J.F., "Effects of Frying Oil and *Houttuyniacordata*Thunb on Xenobiotic-Metabolizing Enzyme System of Rodents," World Journal of Gastroenterology, 2005: 11(3) pp.389-392.

[73] Zheng, H.Z., Dong, Z.H. and She, J., Modern study of traditional Chinese medicine. Xue Yuan Press Beijing China, 1998: 3**,**pp. 2057.

[74] Zheng, W. and Wang, S.Y., Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food chemistry, 2001: 49(11) pp.5165-5170.

[75] Yasir, M., Sultana, B. and Amicucci, M., Biological activities of phenolic compounds extracted from Amaranthaceae plants and their LC/ESI-MS/MS profiling. Journal of Functional Foods, 2016: 26, pp. 645-656.

[76] de Oliveira, V. M. A., Carneiro, A. L. B., Cauper, G. S. D. B. andPohlit, A. M., In vitro screening of Amazonian plants for hemolytic activity and inhibition of platelet aggregation in human blood. ActaAmazonica, 2009: 39(4), pp. 973–980.

[77] Tian, L., Zhao, Y., Guo, C. and Yang, X.,A comparative study on the antioxidant activities of an acidic polysaccharide and various solvent extracts derived from herbal *Houttuyniacordata.* Carbohydrate Polymers,2011*:* 83, pp. 537–544.

[78] Tekle, E.W., Sahu, N.P. and Makesh, M., Antioxidative and antimicrobial activities of different solvent extracts of Moringaoleifera: an in vitro evaluation. International Journal for Scientific and Research Publications, 2015: 5(5), pp. 255-266.

[79] Chiow, K.H., Phoon, M.C. Putti, T., Tan, B.K.H., Chow, V.T., Evaluation of antiviral activities of *Houttuyniacordata*Thunb. extract, quercetin, quercetrin and cinanserin on murine *coronavirus* and dengue virus infection. Asian Pacific Journal of Tropical Medicine,2016: 9(1), pp. 1–7.

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