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EVALUATION OF ANTIOXIDANT ACTIVITY, QUANTITATIVE ESTIMATION OF A POLYHERBAL EXTRACT

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ABSTRACT

Among the various causes of death in the world are degenerative diseases such as heart failure, anaemia, Diabetes Mellitus and stroke, which are generally linked to the oxidative stress. The therapeutic benefit of medicinal plants in the treatment of degenerative diseases is attributed to their antioxidant properties. In the present study crude (PH) extract of nine different medicinal plants are used and were evaluated for their quantitative phytochemicals such as flavonoids, phenols, tannins, carbohydrate, alkaloid, saponin, cardiac glycosides and their antioxidant activities using DPPH, reducing power , hydroxyl radical scavenging, superoxide radical scavenging, Nitric oxide radical scavenging . Total antioxidant activity was done by FTC assay and compared with TBA method. In the present study, results showed that the crude (PH) extract of nine different medicinal plants have found to possess a good antioxidant activity. This activity of PH formulation may be attributed to their free radical scavenging ability. The extent of antioxidant activity of PH was found to be significant.

KEYWORDS

Quantitative phytochemicals, Free radical scavenging, FTC and TBA method, Crude extract and polyherbal (PH) extract.

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INTRODUCTION

Medicinal plants are major part of new pharmaceuticals and health care products. Due to the availability of medicinal plants throughout the world, herbal drugs are being used by 75-80% of world population, especially in developing countries. As reported by World Health Organization (WHO), traditional medicinal plants are the best reservoirs to develop newer pharmaceuticals[1]. Medicinal plants are renewable sources therefore farmers get encouraged to

include them in traditional agriculture[2]. India has highly diverse vegetation and herbal plants which are rich source of bio active compounds[3].

Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects[4]. However, such plants should be investigated to better understand their properties, safety, and efficiency[2].

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and this bio active substances include tannin, alkaloids, carbohydrates, terpenoids, steroids and flavonoids[3,5]. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. This can be derived from barks, leaves, flowers, roots, fruits, seeds[6].

Clinical studies have showed that there is an increased generation of reactive oxygen species such as superoxide anion and hydroxyl radicals in heart failure, which involved in the formation of lipid peroxides, destruction of antioxidative defense system and damage of cell membranes[7]. Therapeutic inventions via suppression of free radical generation and enhancement of endogenous antioxidant enzymes may limit the infarct size and attenuate myocardial dysfunction[8]. The formation of free radicals involved in the pathology of various diseases including diabetes, heart attacks, inflammation, neuro-degenerative diseases, cancer and ageing[9].

Antioxidants may offer resistance against oxidative stress by scavenging free radicals and they prevent diseases. Most of the authors co-related the antioxidant potential of the plant with their phenolic constituents[9,10]. Various synthetic antioxidants are on the use, but they are suspected to be carcinogenic. Natural antioxidants, therefore, have gained importance[11]. DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts. Chromatophore ABTS+ was formed by the reaction between ABTS and potassium per sulphate and reduced to ABTS by the action of antioxidants available in the extracts[11].

Flavonoids and some other phenolic compounds of plant origin have been reported as scavengers of free radicals. Hence, nowadays search for natural antioxidant source is gaining much importance[12]. Reactive oxygen species are produced as a natural byproduct/ intermediates in biological processes in body by the normal oxygen metabolism[13]. It is now accepted that qualitative and quantitative analysis of major bioactive chemical components of a crude drug constituents an important and reliable part of quality control protocol, since any change in quality of the drug directly affects the constituents. Such analysis need to be developed for every aspect of poly herbal extracts and also single herbs[13]. Therefore, researchers focused their interest towards herbal medicines in the treatment of diseases because of their minimal side effects and availability[10].

Number of techniques and methods used to elucidate the active ingredients in the biological resources. Among these, the quantitative analysis is very essential for identifying active metabolites present in the medicinal plants which are important for pharmacological therapeutic action.

MATERIALS AND METHODS

Chemicals required

All chemicals used for this study were high quality analytical grade reagents. The solvents such as ethanol, water and hexane were purchased from S.D. Fine Chemicals Pvt. Ltd, Sigma chemicals, Lobe chemicals, Merck Chemical Supplies, Nice Chemicals and Hi media. All other chemicals used for the study were obtained commercially and were of analytical grade.

Preparation of polyherbal formulation and solvent extraction

Each one gm of a poly herbal (PH) formulation contains equal amount of *Punica granatum(rind), Catharanthus roseus, Gymnema sylvestre, Cissus quadrangularis, Garcinia cambogia, tinospora cordifolia, Terminalia Arjuna, Urginea indica, Ficus racemosa*. The plants were authentified in Botanical survey of India, Coimbatore. 10g of the dried powder of each plant was taken and cold macerated with

hydro-ethanolic solvent with occasional stirring for 3 days.

After 3 days, the suspensions was filtered through a fine muslin cloth and the filtrate was evaporated to dryness at low temperature (<40^0 C) under reduced pressure in a rotary evaporator. The yield of crude extract is called as polyherbal (PH) extract which was found to be 9.64% and were stored in an air-tight desiccator's and used for further analysis.

QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS

Determination of Flavonoid Content

The flavonoid content of the plant extract was determined by a colorimetric method (Xia Liu *et al*., 2005 and Zhishen *et al*., 1999) with minor modification. Different concentration of the plant extract was prepared by diluting the stock solution (4000 µg/ml) with deionized water. Each sample (100 µl) was diluted with distilled water (200 µl). Sodium nitrite (5 %; 30 µl) was added to the samples and then at 5 minutes, aluminium chloride (10 %; 30 µl) and at 6 minutes, sodium hydroxide (1 M; 200 µl) were added to the mixture. Finally, 400 µl of deionized water was added. The absorbance was recorded at 510 nm. Quercetin was used as the standard to calculate the concentrations of flavonoid content and the values were expressed as mg Quercetin equivalents/g of sample.

Determination of Total Phenolic Content

The total phenolic content of crude extract of the plant material was estimated according to the method of Siddhuraju and Becker method (2003). Different aliquots of the extracts were taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against a reagent blank. Total phenolic contents were determined as a Gallic acid equivalent (GAE) based on Folin-Ciocalteau calibration curve using Gallic acid (ranging from 50 to 1000 mg/ml) as the standard and expressed as mg Gallic acid equivalent per gram of dry sample.

Determination of Tannin content

The Tannin was determined by the methods of Van-Burden and Robinson (1981). 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1h in a mechanical shaker. Then filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Determination of carbohydrate content

The total carbohydrate content of crude extract of the plant material was estimated according to the method of Sadasivam (1984).100 mg of sample was hydrolysed in a boiling tube with 5 ml of 2.5 N HCl in a boiling water bath for a period of 3 hours. It was cooled to room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100 ml using distilled water. From this 0.2 ml of sample was pipetted out and made up the volume to 1 ml with distilled water. Then 1.0ml of phenol reagent was added followed by 5.0 ml of sulphuric acid. The tubes were kept at 25-30 ºC for 20 min. The absorbance was read at 490 nm.

Determination of Alkaloid content

The Alkaloid was determined by the methods of Harborne (1973). 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added and allowed to stand for 4h. Then filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid was dried and weighed.

Determination of Saponin content

The Saponin was determined by the methods of Obadoni and Ochuko (2001). 20 g of plant sample were put into a conical flask and 100 cm^3 of 20 %

aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 °C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight.

Determination of Cardiac gylcosides

Cardiac glycoside content in the sample was evaluated using Buljet's reagent as described by El-Olemy et al (1994). 1g of the crude extract was soaked in 10ml of 70% alcohol for 2hrs. and then filtered. The extract obtained was then purified using lead acetate and Na_2HPO_4 solution before the addition of freshly prepared Buljet's reagent (containing 95ml aqueous picric acid + 5ml 10% aqueous NaOH). The difference between the intensity of colours of the experimental and blank (distilled water and Buljet's reagent) samples gives the absorbance and is proportional to the concentration of the glycosides.

ANTIOXIDANT ACTIVITY[14-18]

After the completion of quantitative phytochemical analysis antioxidant activity is been carried by standard procedures.

DPPH (2,2-diphenyl-l-picryl hydrazyl) base free radical Scavenging activity

The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging protocol. DPPH solution (0.004% w/v) was prepared in 95% ethanol. A stock solution of chloroform extract, ethanolic extract and standard ascorbic acid were prepared in the concentration of 10mg/100ml (100µg/ml). From stock solution 2ml, 4ml, 6ml, 8ml and 10ml of this solution were taken in five test tubes respectively. With same solvent made the final volume of each test tube up to 10 ml whose concentration was then 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml respectively. 2 ml of freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes. The reaction mixture was incubated in the dark for 15 min and thereafter the optical density was recorded at 523 nm against the blank. For the control, 2 ml of DPPH solution in ethanol was mixed with 10ml of ethanol and the optical density of the solution was recorded after 30 min. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation

$$\% \text{ inhibition of DPPH Scavenged} = \frac{(\text{A control} - \text{A test})}{(\text{A control})} \times 100$$

Where "A control" is the absorbance of the control reaction and "A test" is the absorbance of the sample of the extracts. IC50values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Ferric reducing antioxidant power (FRAP)

The FRAP assay was performed according to Benzie *et al.,* (1996) with slight modifications suggested by Firuzi and Lancana (2005). FRAP reagent was prepared and kept at 35ºC for 5-10 min prior to use. In a 96 well plate, 175 µl of FRAP was added and the plate was pre read at 593nm. To each well, 25µl of sample solution was added and contents were mixed well using a pipette. Absorbance was measured at 593nm at 4, 15, 30 and 60 min, using methanol as blank. Change in absorbance at different time intervals was then translated in to a FRAP value using a standar trolox curve.

Nitric oxide scavenging activity assay

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964). Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 M sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of chloroform and ethanolic extracts at various concentrations and the mixture incubated at

25ºC for 2hrs. From the incubated mixture 0.5 ml was taken out and added into 1ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated using the same above equation. IC50values denote the concentration of sample, which is required to scavenge 50% of Nitric oxide radicals.

$$\text{Percent inhibition of Nitric oxide radical} = \frac{\text{(Abs of control – Abs of sample)}}{\text{(A control)}} \text{ X 100}$$

Where, Abs (control): Absorbance of the control and
Abs (test): Absorbance of the extracts/standard.

H_2O_2 radical scavenging assay

The ability of the extract to scavenge hydrogen peroxide was determined according to the method given by Ruch *et al.*,(1989).A solution A solution of hydrogen peroxide (2mmol/l) was prepared in phosphate buffer (pH 7.4). Extracts (1–10 µg/ml) were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide and compared with ascorbic acid, the reference compound. IC50 values denote the concentration of sample, which is required to scavenge 50% of H_2O_2 radicals.

$$\text{H_2O_2 Scavenged (\%)} = \frac{\text{(A control – A test)}}{\text{(A control)}} \text{ X 100}$$

Where, Abs (control): Absorbance of the control and
Abs (test): Absorbance of the extracts/standard.

Superoxide radical scavenging assay

Measurement of superoxide radical scavenging activity of the crystals was done by using the standard method (Nishikimi *et al*., 1972) followed by slight modification. The reaction mixture contained 1ml of NBT solution (312µM prepared in phosphte buffer, pH 7.4), 1ml of NADH solution (936µM prepared in phosphte buffer, pH 7.4) and standardized 50 times methanol diluted different extracts of the sample were added. Finally, reaction mixture were accelerated by adding 100µl PMS solution (120µM prepared in phosphte buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25 ºC for 5min and absorbance at 560nm was measured against methanol as control. Percentage of inhibiton was calculated.

$$\text{Percentage of activity} = \frac{\text{Absorbance of (Control – Test)}}{\text{Absorbance control}} \text{ X 100}$$

Hydroxyl radical scavenging assay

Scavenging of the hydroxyl free radical was measured by the method of (Halliwell *et al.*, 1989) with minor changes. All the solutions were prepared freshly .200µl of 2.8mM 2-deoxy-2-ribose, 5µl crude sample, 400µL of 200µM $FeCl_3$, 1.04mM EDTA (1:1V/V), 200µl of H_2O_2 (1.0mM) and 200µl of ascorbic acid (1mM) was mixed to form a reaction mixture. After an incubation period of one hour at 37ºC the extent of deoxyribose degradation was measured by the TBA reaction 1.5ml of 2.8% TCA was added in the reaction mixture and kept for 20min at 100ºC taking Vitamin E as control. Percentage inhibition was calculated as same equation.

TOATAL ANTIOXIDANT ASSAY[18]

Ferric Thiocyanate (FTC) Method

The standard method as described by Kikuzaki and Nakatani (1993). A mixture containing 4 mg of the chloroform and ethanolic extract in 4 ml of 99.5% ethanol (final concentration 0.02%). 4.1ml of 2.52% linoleic acid in 99% ethanol, 8 ml of 0.05M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a vial with screw cap and then placed in an incubator at 400C in the dark separately. To 0.1 ml of this mixture 9.7 ml of 75% ethanol (v/v) and 0.1 ml of 30% ammonium thiocyanate were added. Precisely 3 minutes later the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloric acid was added to reaction mixture; (the absorbance of red color indicated the antioxidant activity) was measured at 500 nm for every 24 hours until the absorbance of the control reached maximum. The control and the standard were subjected to the same procedures as the sample except that for the control, only the solvent was used, and for the standard 4mg of the sample was replaced by 4 mg of Vitamin C.

Thiobarbituric Acid (TBA) Method

TBA method used for evaluating the extent of lipid peroxidation. At low pH and high temperature (1000ºC), melonaldehyde binds with TBA to form a red complex that can be measure at 532 nm. 2 ml of 20% trichloroacetic acid and 2 ml of 0.67% TBA solutions were added to 2 ml of the mixtures containing the sample prepared in the FTC method. This mixture was kept in water bath (1000ºC) for 10 minutes and after cooling to room temperature, was centrifuged at 3000 rpm for 20 minutes. Antioxidant activity was based on the absorbance of the supernatant at 532 nm on the final day of the assay. The percentage of antioxidant activity was calculated by following formulae for both FTC and TBA (Sawarkar H.A.*et al.*, 2009).

$$\text{Percentage Activity} = 100 - \frac{\text{Absorbance of (Control – Test)}}{\text{Absorbance control}} \text{ X } 100$$

RESULTS AND DISCUSSION

Quantitative Phytochemical Analysis

The level of quantitative phytochemical analysis was presented in Table No.1. The plants are found to be a good source of antioxidants.

Flavonoids are the phenolic substances which are the largest group of phenols. They generally occur as a C6-C3 unit linked to an aromatic ring. They are other plant constituents with antibacterial and antifungal properties[19]. The flavonoid content of crude (PH) extract was found to be 20 mg/g.

Phenols are the most widespread secondary metabolites in plant kingdom. This diverse group of compounds has received much attention as potential natural antioxidant, in terms of their ability to act as both efficient radical scavengers and metal chelators. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers[20]. In the present study, the total phenolic content was found to be 31mg/g.

The tannin content of the crude extract was found to be 45 mg/g .Tannins is complex moieties produced by majority of plants as protective substances having wide pharmacological activities. Several reviews are dietary antioxidants were indicated that they have been used since past as tannin agents and reported for astringent, anti-inflammatory, anti-diarrheal, antioxidant and anti-microbial activities[21].

CHO level in the crude extract was depicted as 41 mg/g. Polysaccharides are important natural product in traditional Chinese medicine, possess antioxidant property by which protect cells against ROS, chronic and degenerative diseases[22],[23], reported that presence of various secondary metabolites shows a significant antioxidant property.

The alkaloid content of the crude extract was found to be 23 mg/g Alkaloids which are one of the largest groups of phytochemical in plants having amazing effects on humans and this has led to the development of powerful pain killer modifications[24]. Also studies have showed that alkaloid is capable of reducing headaches associated with hypertension[25].

The saponin content of the crude extract was found to be 16 mg/g Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases[26] for eg, saponins possess both beneficial (cholesterol-lowering) and deleterious (cyotoxic permeabilization of the intestine) properties and also exhibit structure dependent biological activities[27]. Saponins have been found to be potentially used for the treatment of hyperglycaemia[28,29] and exhibit antibiotic properties.

Cardiac glycosides level of the crude was found to be 48 mg/g. cardiac glycosides work by inhibiting the Na+/k+ pump. This causes an increase in the level of sodium ions in the mycocytes, which then leads to a rise in the level of calcium ions. This inhibition increases the amount of Ca2+ ions available for contraction of the heart muscle, improves cardiac output and reduces distention of the heart[30].

Antioxidant Activity

Free radicals are constantly generated resulting in extensive damage to tissues and biomolecules leading to various disease conditions. So the medicinal plants are employed as an alternative source of medicine to migrate the diseases associated with oxidative stress. The results obtained are shown in Table No.2.

The effect of the antioxidant on DPPH is thought to be due to their hydrogen donating ability[31]. The results indicated that the hydroethanolic (PH) extract of the

mixture at the concentration of about 500µg/ml exhibited maximum inhibition, ie: 62.9% whereas the inhibit produced by L-ascorbic acid (standard) was 98.78%. Furthermore, the hydroethanolic (PH) extract at other concentrations ie:100, 200,300,400mg showed marked DPPH scavenging activity in terms of 57.3,57.5,57.8,58.3,61.2% inhibition, respectively. The amount of hydroethanolic extract needed for 50% inhibition of DPPH radical was 580µg/ml which is higher than IC50 value of ascorbic acid 350µg/ml. Hence it is concluded that hydroethanolic extract is approximately as effective as ascorbic acid as an antioxidant.

The results obtained from FRAP values at different time intervals showed 596.8 at 4^{th} min steadily followed an increasing trend to 993.67 at 30^{th} min further reaching at 1116.8 at 60^{th} min shown in Table No.3. The hydroethanolic extract has a significant ability to bring about the reduction of ferric ions in a time dependent manner. At a concentration of 50µg/ml, Daphne Kiusiane has been reported to possess a reducing power of 72%[32]. The reducing power of a compound is related to its electron transfer ability. Reducing power is widely used to evaluate the antioxidant of polyphenols, which exerts antioxidant activity by breaking the free radical chain by donating a hydrogen atom[33]. FRAP value serves as a measure of Fe (II) TPTZ reducing power of the extract.

Nitric oxide is regarded as an important mediator of acute and chronic inflammation, which can easily react with superoxide anion to form a peroxynitrite (ONOO), a potent oxidizing molecule capable of eliciting lipid peroxidation and cellular damage[34-36]. The hydroethanolic (PH) extract was tested for its scavenging potential. The % of inhibition was evaluated at different concentration and it ranges between 100- 500µg/ml. The IC50value was found to be 610µg/ml for the extract as against 480µg/ml of standard. In all, nitric oxide has the ability to exert multiple cytotoxic effects. Any plant with a considerable ability to terminate NO radical's *invitro* would prove to be an important source of natural antioxidant lead molecules.

Hydrogen peroxide is a week oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups[37]. Hydrogen peroxide can cross cell membrane rapidly and once inside the cell, hydrogen peroxide can probably react with Fe^{2+} and possibly CU^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects. It is therefore biologically benefical for cells to control the accumulated hydrogen peroxide. Table No.2 shows that the extract demonstrated hydrogen activity in a concentration manner.

The crude (PH) extract was found to be notable scavenger of SO radical generated by numerous biological and phytochemical reactions[38]. So it can further interact with other molecules to generate secondary ROS either directly or indirectly or prevalently through enzyme or metal catalysed processes[39]. The scavenging activity of the crude (IC50 750µg/ml). The inhibition concentration of 200µg/ml showed 38.68% whereas 500µg/ml shows 54.07%. This clearly indicates the inhibition activity was dose dependent.

Hydroxyl radicals generated in the human body plays a vital role in tissue injury at sites of inflammation in oxidative stress originated diseases. The hydroxyl radical is the most reactive of the ROS which induces severe damage in adjacent molecules[40]. The hydroxyl radical can cause oxidative damage to DNA, lipids and proteins (43). The crude extract showed potent hydroxyl radical scavenging activity. The potential extract inhibits hydroxyl radical mediated deoxyribose damage which was assessed by means of iron (II) dependent DNA damage assay. In the present study the crude the hydroxyl radical scavenging effect of the crude extract in the concentration of 500µg/ml was found to be 79.48% and ascorbic acid was used as a control since it is reported to be significantly effective inhibition of hydroxyl radicals, which shows 84.59% scavenging effect at same concentration. The IC50 value of ascorbic acid was found at the conc. of 380µg/ml and extract is 540µg/ml respectively.

TOTAL ANTIOXIDANT ASSAY

FTC and TBA method

Antioxidant activity of the crude extract was measured using FTC as shown in Figure No.1. The inhibition percentage of FTC was found to be 80.4% of the crude

extract. In the FTC test, it determines the amount of peroxide produced during initial stage of lipid peroxidation, whereas ferric ion was formed upon reaction of peroxide with ferrous chloride. The ferric ion will then unite with ammonium thiocyanate producing ferric thiocyanate, a red colored substance. The darker the colour, the higher will be the absorbance. As shown in fig, the extract delayed the oxidation of linolenic acid, beaded on low absorbance values and exhibited higher antioxidant activity. This reduction is due to malondealdehyde (MDA) which is not stable. Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative stress. Especially linolineic acid and Arachidonic acid are the targets of LPO[44]. In the present study the % of inhibition as the gradual increase in the antioxidant activity by TBA method (Figure No.2). FTC is used to measure the secondary product of oxidation such as aldehyde and ketone. MDA is one of the TBARS and products of lipid peroxidation, studies widely as a marker of oxidative stress, and as an index of lipid peroxidation[41].

Table No.1: Quantitative Phytochemical Analysis

S.No	Parameters	Quantity (mg/g)
1	Flavonoids	20
2	Total Phenol	31
3	Tannins	45
4	Carbohydrate	41
5	Alkaloids	23
6	Saponins	16
7	Cardiac glycosides	48

Table No.2: Antioxidant activity of a Polyherbal Extract

S.No	Concentration of the polyherbal extract	Percentage scavenging activity								
		Dpph	Rutin	No	Mannitol	H_2o_2	So	Quercetin	Ho	Ascorbate
1	100	57.3	97.27	46.49	55.17	58.13	35.78	56.38	51.88	62.87
2	200	57.5	97.95	46.51	61.15	59.25	38.68	62.23	53.34	68.12
3	300	57.8	98.10	46.55	67.10	60.40	44.14	72.17	61.15	76.88
4	400	56.5	98.18	55.34	76.60	62.93	49.53	81.84	76.67	82.70
5	500	62.9	98.78	76.55	84.60	67.90	54.07	91.11	79.48	84.59
6	Ic50	580	350	610	480	520	750	330	540	380

Table No.3: Frap Assay

S.No	Time (min)	Concentration	Frap Values of Extract (µm TE/mg)
1	0	0	0
2	4	200	680
3	15	400	845
4	30	600	996
5	45	800	1105
6	60	1000	1230

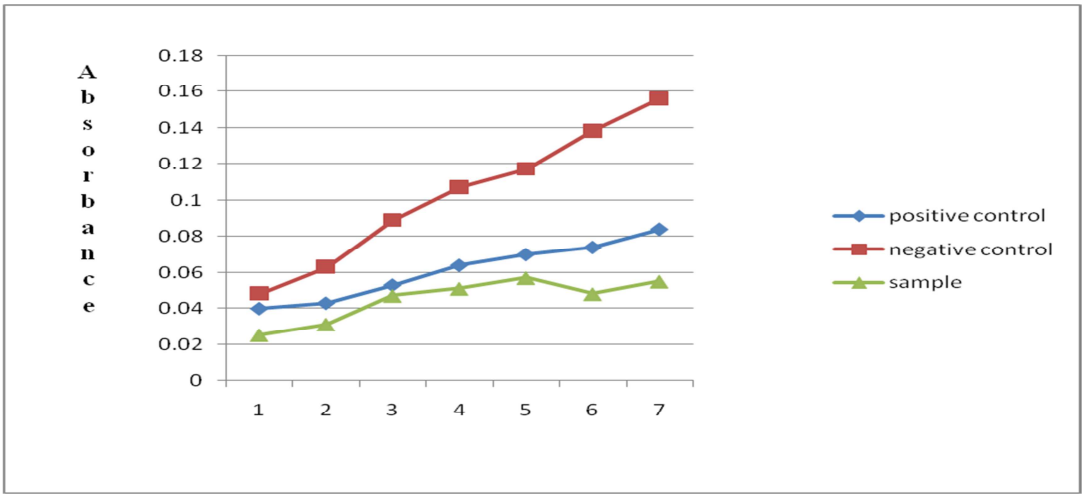

Figure No.1: Total Antioxidant Activity by FTC Method

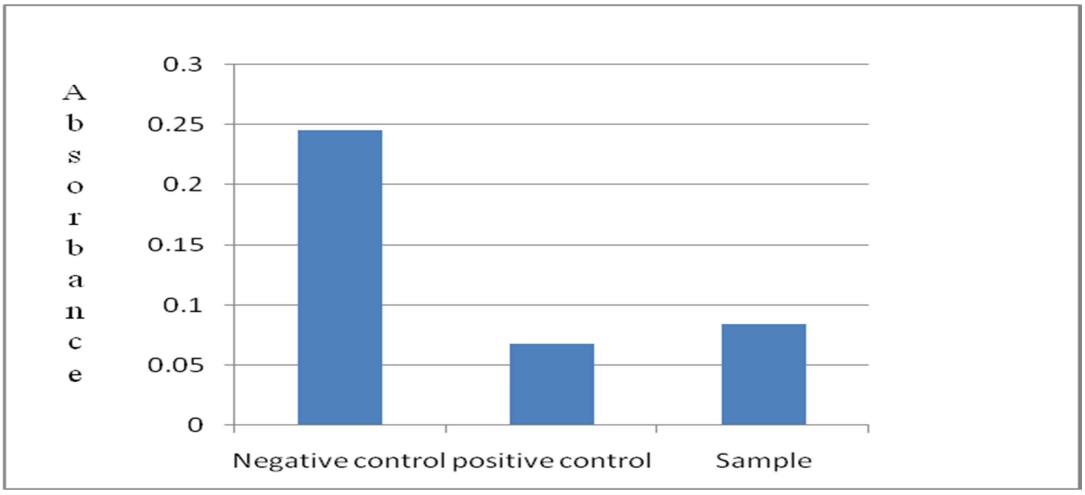

Figure No.2: Total Antioxidant activity by TBA Method

CONCLUSION

The results obtained from this study strongly suggest that the crude has a significant antioxidant activity, could serve as an easily accessible item of natural rich antioxidant food which may enhance the immune system against oxidative damage, or it may be utilized as a potential source of therapeutic agent.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Phillipson J D, Wright C W. Plants with Antiprotozoal Activity: Tease and Evans, Pharmacognosy, *WB Saunders Company, London*, 14th edition, 1996, 612.
2. Arunkumar S, Muthuselvam. Analysis of phytochemical constituents and antimicrobial activities of *aloevera L.* against clinical pathogens, *World J. Agril. Sc.*, 5(5), 2009, 572-576.
3. Edoga H O, Okwu D E, Mbaebie B O. 2005. Phytochemicals constituents of some Nigerian medicinal plants, *Afr. J. Biotechnol.*, 4(7), 2005, 685-688.

4. Dewick P M. Tumor inhibition from plants, *Tease and Evans*, 1996.
5. Mann J. Secondary Metabolism, *Oxford University press, London,* 1978, 154.
6. Rajan S, Mahalakshmi S, Deepa V M, Sathya K, Shajitha S, Thirunalasundari T. Antioxidant potentials of *Punica granatum* fruit rind extracts, *Int j pharm pharm sci,* 3(3), 2011, 82-88.
7. Alla F, Zannad F, Filippatos G. Epidemiology of acute heart failure syndromes, *Heart Fail Rev,* 12, 2007, 95.
8. Nakamura T, Nishi H, Kokusenya Y, Hirota K, Miura Y. Mechanism of antioxidative activity of fluvastatin determination of the active position, *chem pharma bull*, 48, 2000, 237.
9. Santoyo S, Jaime L, Herrero M, Senorans F, Cifuentes A, Ibanez E. Functional characterization of pressurized liquid extracts of *Spirulina platensis, European Food Research Technology,* 224, 2006, 75-81.
10. Nishikimi M, Rao N A, *et al.* "The occurrence of superoxide anion in the reaction of reduced Phenazine methosulphate and molecular oxygen", *Biochem Biophys Res. Commun.,* 46, 1972, 849-53.
11. Jaina N, Goyala S, Ramawata K G. Evaluation of antioxidant properties and total phenolic content of medicinal plants used in diet therapy during postpartum healthcare in Rajasthan, *Int j pharm pharm sci,* 3(3), 2011, 248-253.
12. Mukherjee P K. Evaluation of Indian traditional medicine, *Drug information Journal*, 35, 2001, 631-40.
13. Nakamura T, Nishi H, Kokusenya Y, Hirota K, Miura Y. Mechanism of antioxidative activity of fluvastatin determination of the active position, *chem pharma bull*, 48, 2000, 237.
14. Harborne J B. Phytochemical methods. *London, Chapman and Hall, Ltd.,* 1973, 49-188.
15. Obadoni B O, Ochuko P O. Phytochemical studies and comparative efficacy of crude extracts of some homeostatic plants in Edo and Delta States of Nigeria, *Global Journal of Pure Applied Sciences*, 8, 2001, 203-208.
16. Van-Burden T P, Robinson W C. Formation of complexes between protein and Tannin acid, *Journal of Agricultural Food Chemistry,* 1, 1981, 77.
17. Xia L, Yuangang L, Yujie F, Liping Y, Chengbo G. Antimicrobial activity and cytotoxicity towards cancer cells of *Melaleuca alternifolia* (tea tree) oil, *European Food Research and Technology*, 229, 2005, 247-253.
18. Krishnaveni S, Balasubramanian T and Sadasivam S. Phenol Sulphuric acid Method, *Food Chemistry,* 15, 1984, 229.
19. El-Olemy M M, Al-Muhtadi F J and Afifi A F A. Experimental phytochemistry: A laboratory manual, *King Saud University Press, Saudi Arabia,* 1994, 21-27.
20. Garrat D C. The Quantitative Analysis of Drugs, *Chapman and Hall, Japan.*, 1964, 456.
21. Kikuzaki H and Nakatani N. "Antioxidant effects of some ginger constituents", *Journal of Food Science*, 58, 1993, 1407-1410.
22. Sawarkar H A, Khadabadi S S, Wandhare M D, Farooqui I A and Deokate U A. "The antioxidant activity of the leaves of *Barleria Grandiflora Daiz.*(Acanthaceae)", *Journal of Herbal Medicine and Toxicology,* 3(2), 2009, 63-66.
23. Ruch R J, Cheng S J, Klaunig J E. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea, *Carcinogenesis,* 10, 1989, 1003-1008.
24. Narayanasamy K, Ragavan B. Phytochemical and mineral screening of *Zanthozylum Tetraspermum* (W and A).
25. Narayanasamy K, Ragavan B. *Invitro* antioxidant activity of *Zanthozylum Tetraspermum* (W and A) stembark, *International Journal of engineering science and technology,* 4, 2012, 155-162.
26. Suresh G Killedar, Harinath N More. Estimation of tannins in different parts of

mymecylon Umbellatum Burm, *Journal of pharmacy research*, 3, 2010, 554-56.
27. Bin. Li.Xan-Jun, Meng.Li-wei-sun. Isolation of chemical characterization and *invitro* antioxidant activities of polysaccharides from *Aconiyum Coreanum*, *Journal of Medicinal Plants Research*, 6, 2012, 876-73.
28. Song H F, Zhang Q B, Zhang Z S, Wangs. Invitro antioxidant activity of polysaccharides extracted from Bryopsis plumose, *Carbohydrate polymers*, 80, 2010, 1057-58.
29. Kam P C, Liew A. Traditional Chinese herbal medicine and anaesthesia, *Anaesthesia*, 57, 2002, 1083-89.
30. Ayitey- Smith E, Addae-Mensah I. Phytochemical, Nutritional and medicinal properties of some leafy vegetables consumed by Edo people of Nigeria, *W.Afr.J.Pharmacol.Drug Res.*, 4, 1977, 7-8.
31. Mir M A, Sawhney S S, Jassal M M S. Qualitative and quantitative analysis of phytochemical of Taraxacum Officinale, Wudpecker, Journal of pharmacy and pharmacology, 2(1), 2013, 001-005.
32. Valko M, Leibfritz D, Moncol J, Gonin M T D, Mazur M, Telsor J. Free radical and antioxidants in normal physiological functions and human diseases, *Int J Biochem cell Biol*, 39, 2007, 44-84.
33. Gutteridge M C. Reactivity of hydroxyl and hydroxyl like radicals discriminated by release of Thiobarbituric acid reactive materials from deoxy sugars, nucleosides and benzoate, *Biochem J*, 224, 1984, 761-67.
34. Osagie A U, Eka O U. Mineral elements in plant foods: In:Nutritional quality of plant foods, *Ambik press., Benin city, Edo state, Nigeria*, 1998, 8,14,43 and 86.
35. Olaleye M T. Cytotoxicity and antibacterial activity of Methanolic extract of *Hibiscus Sabdariffa*, *J.Med.Plants Res.*, 1(1), 2007, 009-013.
36. Malenow M R, MC.Laughlin P, Kohler G O, Livingstone A L. Alfalfa Saponins: A family of substances potentially useful for the treatment of hypercholesterolemia, *J.Clin.Res*., 25, 1977, 974-79.
37. Krishnaiah D, Devi T, Bono A, Sarbatly R. Studies on phytochemical constituents of six Malysian medicinal plants, *Journal of Medicinal Plants Research*, 3(2), 2009, 067-072.
38. Baumann J, Wurn G, Bruchlausen F V. Prostaglandin synthetase inhibiting O2- radical scavenging properties of some flavonoids and related phenolic compounds. Deutsche pharmakologische gesellschaft abstracts of the 20th spring meeling, Naunyn - Schmiede bergs, *Arc. Pharmacol*, 307, 1979, R1-77.
39. Beckman J S, Beckman T W, Chen J, Marshall P A, Freeman B A. Apparent hydroxyl radical production by peroxynitrite implication for endothelial injury from nitric oxide and superoxide, *Proceedings of National Academy of science USA*, 87, 1990, 1620-24.
40. Radi R, Beckman J S, Bush K M, freeman B A. Peroxynitrite - induced membrane lipid peroxydation: the cytotoxic potential of superoxide and nitricoxide, *Archieves of Biochemistry and Biophysics*, 288, 1991, 481-87.
41. Govindarajan R, Vijayakumar M, Rawat A K S, Shanta M. Free radical scavenging potential of picorrhiza Kurroa Royle Ex Benth, *Ind J Exp Biol*, 41, 2003, 875-79.

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