Hawraa Saad Al-Kawaz and Lamia AM AL-Mashhady. / International Journal of Pharmacy & Therapeutics, 7(1), 2016, 31-41.
e- ISSN 0976-0342
Print ISSN 2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

EVALUATION OF THE PHYTOCHEMICAL CONSTITUENTS AND OXIDANT – ANTIOXIDANT STATUS FOR *ACTINIDIA DELICIOSA* EXTRACTS

Hawraa Saad Al-Kawaz and Lamia A. M. AL-Mashhady*

Department of Chemistry, College of Science, University of Babylon, Hilla, Iraq.

ABSTRACT

This research focuses on the phytochemical study of ethanolic extract (70%), aqueous extracts (cold, hot) and juice of *Actinidia deliciosa*. The checking of phytochemical constituents involved the qualitative and quantitative analysis. The results for qualitative tests explained that the ethanolic extract and juice contains more amounts of active components such as flavonoids, alkaloids, terpenoids, glycosides and amino acids or primary and secondary amines rather than cold extract and hot extract, also the quantitative analysis illustrated that the ethanolic extracts and juice contains more amounts of flavonoids, and alkaloids because the ethanolic extracts and juice still have high yield from all active components and this result may be due to the type of solvent in the ethanolic extract and fruit juice are nutritious contain more active compounds and due to the increased healthy life style becoming more popular drinks. The Thiobarbituric Acid (TBA) reactive substances assay is a simple and quick assay for the assessment in plants of lipid peroxidation, in which Malondialdehyde (MDA) was evaluated. The ethonolic extract show high level of MDA than aqueous extracts and juice. Another type of antioxidant measured was vitamin E, a protective agent against oxidative stress. Total phenolic content was estimated by employing folin -Ciocalteu reagent and the results show the ethanolic extract contain large amount of total phenolic than other extracts. Also study the antioxidant activity by using DPPH (2,2-diphenyl-1-picrylhydrazyl) as stable free radical and the result illustrated the ethanolic extract have large percent of inhibition DPPH compared with other extracts and juice.

Key Words:- *Actinidia deliciosa*, Phytochemical constituents, Vitamin E, Malondialdehyde, Total phenolic compounds, Alkaloids, DPPH, Egg yolk.

INTRODUCTION

Plants supply a diversity of resources that contribute to the essential needs of both human being and animals like eating, clothing and shelter. Among plants of economic importance are medicinal plants. Medicinal and aromatic plants have played vital role in relieving human tribulation (Soni and Singhai, 2012). Over three - quarters of the world population rely mainly on plants and plant extracts for health keeping. (Wankhade, 2014). Many medicinal plants consist of great amounts of antioxidants other than vitamin C, vitamin E, and carotenoids (Javanmardi *et al*., 2003). Any compound exists in plants is famed as phytochemicals (the chemicals exist in plants according to Greek term Phyton). Phytochemicals are plant chemicals which are non-nutrient in nature but have good preventive action versus certain illness (Ali *et al*., 2014). Phytochemicals are essentially split into two groups that are primary and secondary metabolites. Primary metabolites include general sugar, amino acids, proteins and chlorophyll while secondary metabolites consist of

Corresponding Author

Hawraa Saad Al-Kawaz
Email:- hawrahsaad@yahoo.com

alkaloids, flavonoids, tannins etc., (Dhongade and Chandewar, 2013). The medicinal plant used in this research is *Actinidia deliciosa* which is famous as the king of fruits because of its remarkable highest vitamin C content and balanced nutritional composition of minerals, dietary fibre and different health - beneficial metabolites (Huang *et al*., 2013). Another name of *Actinidia deliciosa* as well known as green kiwi fruit is a member of the Actinidiaceae family. It is a member of the order of scaling woody vines. The genus Actinidia consist of kind with eating fruits (Halimoon and Abdul Hasan, 2010).

Kiwifruit grows of course in different regions. It has been firstly cultivated in the tropics and subtropics in countries like as New Zealand, Chile, France and Japan. (Bursal and Gülçin, 2011). Kiwifruit is one of the most public fruits worldwide, and it has different biological properties, inclusive antioxidant, anti-allergic, and cardiovascular protective effects. The peel of kiwi fruit, which is a secondary of processing, is a good source of flavonoids (Yang *et al*., 2013). Plants in general and fruits, especially, have various compounds with antioxidant properties, which involve ascorbic acid, carotenoids and polyphenols (Park *et al*., 2013). Kiwi fruit is a highly nutritious fruit due to its rise level of vitamin C and its strong antioxidant including carotenoids, phenolics, flavonoids and chlorophyll. Kiwi fruit is a wealthy source of vitamins E, fructose, galactose and minerals, it's contains isoflavones, flavonoids, which are important phytochemical in kiwi extract and act the main type of phytosteroge (Shehata and Soltan, 2013). The large amount of dietary fiber in kiwi fruit helps in decreasing the probability of colon cancer. Kiwi's antioxidant properties help in keeping the body versus free radicals. The flavonoids existing in kiwi fruits protect the cells from oxidative hurt and in turn, help in the DNA from mutation and injury (Nagib, 2013).

Scientific classification of the plant
Kingdom: Plantae
Order: Ericales
Family: Actinidiaceae
Genus: Actinidia
Species: A. deliciosa
Binomial name: *Actinidia deliciosa* (Lawrence, 1951)

MATERIALS AND METHODS

Collection of plant samples

The plant materials were taken from local markets in Hilla that native to southern China. The fruit plants were washed in tap water and removed the peel, then cut into small pieces used to make the ethanolic extracts, aqueous extracts and juice that analyzed.

Preparation of the Extracts

The descent was made by weighting 450 grams of the plant in 1L of ethanol (70% v/v) and using an electric blender to prepare the mixture. The mixture was heated at 45°C using the extraction apparatus for two hours, then filtered by filter paper and evaporated at 40°C up to one third of the initial volume. The remaining solvent was completely evaporated at 40°C, using a hot air oven. In the same way, 450 gm of the fruit in 1L of deionized water only.The solution was heated at 60°C to make hot extract for one hour to purpose of comparison between the phytochemical constituents of aqueous extract with ethanolic extract (Sharma *et al*., 2010). To make the cold extract 450 grams of the plant were weighted and mixed with 1L of deionized water only,then shake well for one hour in electrical shaker.The solution was filtered by using filter paper and the filtrate was kept in the refrigerator to use later (Peter *et al*., 2015).

Preparation of the juice

The juice was made by weighting 450 grams of the fruit and using an electric blender to make mixture, then separated out by using filter paper. The filtrate was kept in the refrigerator to use afterwards.

Phytochemical analysis

Chemical tests were organized on the ethanolic extracts (70%), aqueous extracts and juice of the plant sample using standard methods.

Qualitative analysis of Phytochemical constituents

Test for Alkaloids

Take 0.5 grams of the sample and was defatted with 5% diethyl ether for 15 min. The defatted sample was extracted for 20 minutes with 5 ml of aqueous hydrochloric acid on a boiling water bath. The resulting mixture was centrifuged for 10 minutes at 3000 rpm. 1 ml of the filtrate was treated with a few drops of Dragendroff's reagent and a second 1ml with Mayer's reagent and turbidity was observed (Chinedu *et al*., 2014).

Test for amino acids or primary and secondary amines

One ml of each sample of the plant was boiled for a few minutes in a boiling water bath with 1% of the freshly prepared ninhydrin solution. The appearance of blue-violet color was indicated in the presence of amino acids or primary and secondary amine (Al-Temimi and Al-Mashhedy, 2015).

Test for Anthroquinones

A half gram of each sample was shaken with 10 ml of benzene and was filtered. 0.5 ml of 10 % ammonia

solution was added to the filtrated then the mixture was shaken well and appear violet color in the layer phase indicated the presence of the anthroquinone (Olajide *et al*., 2012).

Test for Flavonoids

Take 0.2 gm of each sample and heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4ml of the filtrated was shaken with 1ml of dilute ammonia solution and observed a yellow coloration indicated the presence of the flavonoids (Anowi *et al*., 2013).

Test for glycosides

Have 1ml of concentrated sulfuric acid and was set in a test tube, 5ml of each sample were mixed with 2ml of glacial acetic acid containing 1 drop of ferric chloride. The over mixture was added to 1ml of concentrated sulfuric acid, so that the concentrated sulfuric acid was underneath the mixture. If glycoside, is found in the sample a brown ring will appear (Mir *et al*., 2013).

Test for Phlobatanins

For each plant sample (extracts, juice) 1% of aqueous hydrochloric acid was added and each plant sample was boiled. Appearance the red colored precipitate indicates a positive result (Edeoga *et al*., 2005).

Test for Saponins

A half grams of each sample were shaken with distilled water in a test tube and it was warming in a water bath and the persistent of froth indicates the entity of saponins (Zohra *et al*., 2012).

Test for Tannins

A half gram of each sample was boiled with 20 ml of distilled water in a test tube and then filtrated. A few drops of 0.1% ferric chloride were added and observed for blue-black coloring (Rashid *et al*., 2013).

Test for Terpenoids

Five ml of each plant sample were mixed with 2 ml of chloroform in a test tube, then 3 ml of concentrated sulfuric acid was carefully added to the mixture to form a layer. An interface with a reddish brown coloring was indicated the presence of terpenoids (Krishnaiah *et al*., 2009).

Quantitative analysis of phytochemical constituents

Alkaloids Determination

An ethanolic extracts, aqueous extracts and juice were weighted at 0.15 gm and taken in a test tube, then 5ml of 20% acetic acid in ethanol was added and leave to stand for 4 hours. This was centrifuged and the supernatant was intensive using a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract till the precipitation was complete. The full solution was pliable to settle and the precipitate was collected by filtration and weighted (Aliyu *et al*., 2008).

2 Flavonoids determination

Ten gm of each plant samples were extracted constantly with 100 ml of 80% aqueous methanol at room temperature. The complete solution was filtered through Whitman filter paper no. 42 (125 m). The filtrate was later converted into a crucible and evaporated into dry and weighed to a constant weight (Sivapalan, 2015).

Saponin Determination

Twenty gm of each sample was put into a conical flask and 100 ml of 20 % aqueous ethanol was added. The samples were made hot on top of hot water bath for 4 hours with continuous stirring at 55 °C. The mixture was filtered and the remains re-extracted with another 200 ml of 20 % ethanol. The combined extracts were reduced to 40 ml over a water bath at 90 °C.

The concentrate was moved into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken forcibly. The aqueous layer was fully recovered while the ether layer was ignored. The purification procedure was repeated, 60 ml of n-butanol was added. The combined normal-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The residual solution was heated in a water bath. After evaporation the samples were dry to a constant weight and saponin content was studied as a percentage (Rukmini *et al*., 2015).

Tannin Determination

All samples were weighted at 0.1 gm and 10 ml of distilled water was added. The solution was shaken for 1 hour in a mechanical shaker. This was centrifuged and 1ml of the clearance was pipped out into a tube and mixed with 200 µl of 0.008 M potassium ferrocyanide and 0.1 M ferric chloride in 0.1 N hydrochloric acid. The absorbance was measured with a colorimeter at 670 nm wavelength, within 10 min. A blank sample was ready with the reagent and distilled water and the absorbance taken in the identical wavelength (Prasad *et al*., 2012).

Oxidant – antioxidant system:

Some biochemical measurements were done using standard methods:

Determination of Malondialdehyde (MDA)

The principle of this method was based on spectrophotometric measurement of the color, occurred during the reaction between thiobarbituric acid and malondialdehyde yielding pinkish red chromogen with an absorbance at 532 nm. 150 µl of extracts sample and juice was put in the test tube, then added 1 ml of 17.5% TCA (17.5% in distilled water, w/v) and 1 ml of 0.6 % TBA (0.6% in distilled water, w/v). All tubes were mixed by vortex, incubated in boiling water bath (80°C) for 15 minutes, and then left to cool (at 25°C). Finally, 1ml of 70% TCA (70% in distilled water, w/v)**.** The mixture was allowed to stand at room temperature for 20 minutes and centrifuged at 450 Xg for 15 minutes. The absorbance of all tubes was measured clear supernatant at 532 nm using spectronic 21 D**.** For the blank 150 µl of distilled water was used in place of the extract (Guidet and Shah,1989).

$$\text{The concentration of MDA (µmole /L)} = (A_{sample} / L * €) * D$$

Where :
L : light path (1 cm)
€ : Extinction coefficient ($1.56 * 10^5 M^{-1} cm^{-1}$)
D : Dilution factor
D = 1ml (volume used in reference) / 0.15 ml (volume used in sample) = 1 / 0.15 = 6.7

Lipid peroxidation assay

A modified thiobarbituric acid-reactive species (TBARS) assay was used to measure the lipid peroxide formed, using egg-yolk homogenates as lipid rich media. Malondialdehyde (MDA), a secondary product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA), yielding a pinkish red chromogen with an absorbance maximum at 532 nm. Egg homogenate (0.5 ml, 10% in distilled water, v/v) and 0.1 ml of extract were mixed in a test tube and the volume was made up to 1 ml, by adding distilled water. Finally, 0.05 ml FeSO4 (0.07 M) was added to the above mixture and incubated for 30 min, to induce lipid peroxidation. Thereafter, 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 ml of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl sulphate) and 0.05 ml 20% TCA were added, vortexed and then heated in a boiling water bath for 60 min. After cooling, 5.0 ml of 1-butanol was added to each tube and centrifuged at 3000 rpm for 10 min.

The absorbance of the organic upper layer was measured at 532 nm. For the blank 0.1 ml of distilled water was used in place of the extract (Pandey *et al*., 2007). The percent of Lipid peroxidation was calculated by using the equation as below (Poorna *et al*., 2012).

$$\text{Lipid peroxidation \%} = 100 - \left[\frac{A_1}{A_2} * 100\right]$$

A_1 : Absorbance value in the presence of extract, juice.
A_2: Absorbance value of the fully oxidized control.

Determination of Vitamin E:

Proteins are precipitated by an equal volume of absolute ethanol. The α,α-dipyridyl is added to an aliquot of the upper layer to determine the principle interfering substance at 460 nm. At this time the ferric chloride was added to the system to change the color of the solution which is measured at 520 nm. 500 µl of all extract and juice was put in a test tube, then 500 µl of ethanol was added to sample and 500 µl of xylene was added. All tubes were mixed for 10 min, centrifuged for 10 min at 450 xg. 800 µl of xylene was added then 800 µl of α,α-dipyridyl (1.2 gm in a liter of n-propanol) was added to the solution.Taken out to read the absorbance of the sample at 460 by using spectronic 21 D. Finally, 200 µl of $FeCl_3.6H_2O$ (1.2 gm in a liter of ethanol) was added to the solution. The absorbance of all tubes was measured at 520 nm using spectronic 21 D. The concentration of vitamin E was calculated as below (Hashim and Schuttringer, 1966).

$$\text{Vitamin E (mg/L)} = \frac{A_{\text{for sample in 520 nm}} - A_{\text{for sample in 460 nm}}}{A_{\text{for standard in 520 nm}}} * 10$$

Determination of Total Phenolic Content:

The assay depends on the reduction of Folin-Ciocalteu reagent by the phenolic compounds. The reduced Folin-Ciocalteu reagent was produced blue color absorbed at 765 nm. Folin - Ciocalteu reagent is a mixing of phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic ($H_3PMo_{12}O_{40}$) acids, is reduced to blue oxidation of tungstene (W_8O_{23}) and molybdene (Mo_8O_{23}) through phenol oxidation. This reaction occurs down alkaline condition provided by sodium carbonate. The intensity of blue color reflects the amount of phenolic compounds, which can be measured using a spectrophotometer (Almey *et al*., 2010).

The amount of total phenolics in plant extracts and juice was estimated with the Folin-Ciocalteau reagent. To 0.50 ml of each sample (three replicates), 2.5 ml of 1/10 dilution of Folin-Ciocalteau's reagent and 2 ml of Na_2CO_3 (7.5% w/v) were added and incubated at 45 °C for 15 min. For the blank 0.5 ml of distilled water was used in place of the sample. The absorbance of all samples was measured at 765 nm using a Spectronic 20 D spectrophotometer (Zahin *et al*., 2009). The total phenolic content of the extracts and juice was determined by using the equation as below and using tyrosine as standard

instead of gallic acid, slightly modified (Zendehbad *et al*., 2014).

$$T = \frac{C * V}{M}$$

Where :

T : Total phenolic content mg/g of plant extract in tyrosine.

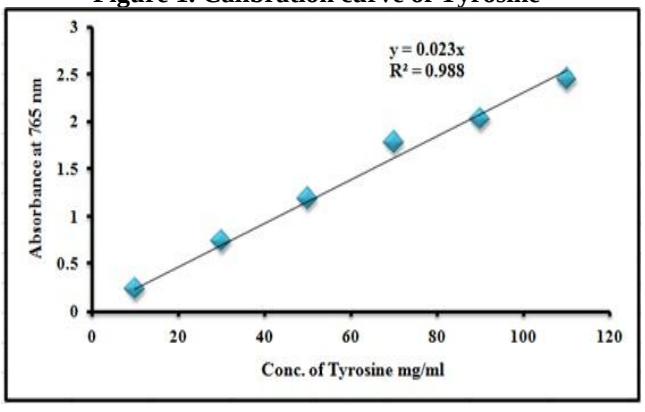
C: Concentration of tyrosine from the calibration curve mg/ml.

V : Volume of the extract in ml.

M : Weight of pure plant in mg.

Tyrosine was used as a standard compound and the total phenols were calculated as mg/g tyrosine equivalent using the standard curve equation show in Figure (1), y = 0.023x, R^2= 0.988, Where y is absorbance at 765 nm and x is total phenolic content in the extracts and juice of *Actinidia deliciosa*

Figure 1. Calibration curve of Tyrosine



DPPH Radical Scavenging Assay:

DPPH (2,2-diphenyl-1-picrylhydrazyl) the stable free radical has been used vastly for the limitation of major anti-oxidant activity DPPH after agreeing electron or hydrogen radical, is converted into stable DPPH-H shape. When this transmutation happens, deep violet color of DPPH change into yellow color. Unconverted DPPH is detected by UV spectrophotometer at 517 nm (Asadujjaman *et al*., 2013). The 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of the sample. After 30 min the absorbance was measured value at 517 nm. Ascorbic acid was utilized as the standard compound.

The lowering in absorbance of the reaction mixture indicated large free radical-scavenging activity. All the tests were performed in triplicate. The inhibition percentage was studied by using the equation as below (Thirunavukkarasu *et al*., 2011).

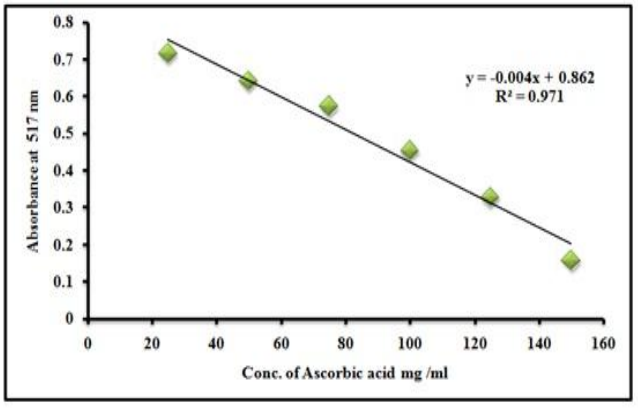
$$\text{Inhibition \%} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} * 100$$

Where :

$Abs_{control}$: Absorbance of the control solution (DPPH with ethanol)

Abs_{sample} : Absorbance of the sample (extracts, juice) solution.

Figure 2. Absorbance of DPPH radical solution prepared in ethanol



RESULTS AND DISCUSSION

Phytochemical analysis is very helpful in the estimation of some active biological components of medicinal fruit and plants. *Actinidia deliciosa* extracts and juice showed positive results for all constituents analyzed, except for anthroquinones, phlobatanins, saponins and tannins as shown in Table (1) and Figure (3).

The medicinal value of these plants lies in close to chemical substances that create a definite physiological action on the human body (Ghumare *et al*., 2014). The minor metabolites such as alkaloids, flavonoids, terpenoids, glycosides, and phenols in plant materials, product the medicinal effect when they are utilized in the conventional medical practice (Vinotha *et al*., 2013). From these secondary metabolites alkaloids and its derivatives, which played significant role in analgesic, antispasmodic and bactericidal activities (Abd Elslam *et al*., 2013). One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms like bacteria (Singh *et al*., 2013). Nitrogen is one of the main nutrients necessary to helpmate perfect plant up growth plants spend nitrogen to form amino acids needed in the forming of protein (Majaw and Moirangthem, 2009). The presence of flavonoids are class of secondary plant metabolites with significant antioxidant and chelating properties. The antioxidant activity of flavonoids depends on the frame and substitution type of hydroxyl groups (Stankovic *et al*., 2011). Flavonoids are free radical

scavenger, super antioxidant and strong water soluble, which prevents oxidative cell injury and have strong anticancer activity. Flavonoids apart from their antioxidant protection effects, prevent the initiation, promotion and progression of tumor (Adamu *et al*., 2013). Also the presence of glycosides is known to lower the blood pressure, according to many reports (Uma and Sekar, 2014). In addition, glycosides play a role in the therapy of failing heart disorders and are known to show helpful effects on cardiac arrhythmias (Vaishali *et al*., 2013). The presence of terpenoids acts as strengthen the skin by increasing the concentration of antioxidants in wounds and restored inflamed tissue by increasing blood supply (Krishnaiah *et al*., 2009).

The presence of higher terpenoids that own carboxylic acid group could also be responsible for the action of the organic extracts (Njoku and Obi, 2009). Terpenoids are reported to have anti-inflammatory, anti-viral, anti-malarial, restraint of cholesterol synthesis and anti-bacterial (Wadood *et al*., 2013). All these phytochemicals posses better antioxidant activities and has been reported to show multiple biological effects, including anti – inflammatory, antitumor activities (Sharma *et al*., 2011). The result of quantitative analysis of *Actinidia deliciosa* extracts and juice (Table 2) shown the presence of alkaloids and flavonoids on different quantities in all extracts, and absence of saponins and tannins and these results show that ethanolic extract is better than hot extract, cold extract and juice. This is due to increase the efficiency of extraction depends on the nature of the solvent.

The results in Table (3) and Figure (5) shown the ethanolic extract gives more value of MDA in comparing with other extract and juice depending on the type of solvent.This means the ethanolic extract can release more lipid compounds compare with another extraction because the Thiobarbituric Acid (TBA) reactive substances, check is an easy and a fast method for the estimation of MDA concentration in plants. In this method, MDA reacts with TBA to form a pink pigment. In plant tissue, however, generated compounds (anthocyanins and carbohydrates) may interfere with measurements at the wavelength 532 nm and provide a false positive reaction with TBA. To prevent the decomposition of lipid peroxides during the analysis, an inhibitor of the lipid peroxidation called butylated hydroxytoluene is added to the sample (Sochor *et al.*, 2012,Wang *et al*., 2013).

As were as the result shown in Table (4) show the ethanolic extract gives more value of lipid peroxidation in comparing with other extract and juice depending on the type of solvent. The procedure of lipid peroxidation has been suggested to proceed by a free radical series reaction, which has been connected with cell injury in bio membranes.The harm has been shown to rapid various diseases such as cancer, cardiovascular diseases and diabetes. Incubation of brain, liver and egg yolk homogeneities in the existence of $FeSO_4$ causes a significant rise in lipid peroxidation (Badmus *et al.*, 2011).

While the result of vitamin E determination shown in The Table (5) and Figure (6). Vitamin E is a term that includes a group of strong, lipid-soluble, chain-breaking antioxidants (Brigelius-Flohe and Maret,1999). The result shown as above the ethanolic extract gives more value of vitamin E in comparing with other extract. The role of vitamin E is as an antioxidant is dependent upon its ability to break radical-propagated chain reactions. As a result, the formation of the tocopheroxyl radical, the single electron derivative of vitamin E, is an inherent part of any vitamin E based, antioxidative reaction. Previous studies have demonstrated that α-tocopherol inhibits LDL oxidation and decreases the release of reactive oxygen species (Anand *et al*., 2003).

The total phenolic contant illustrated that ethanolic extract have more phenolic compound compare with other extracts and juice that may be due to nature of the solvent. Polyphenols are the main plant compound and are usually organized in both edible and inedible plants and they have been reported to have many biological effects, including antioxidant activity (Khalili *et al*., 2013). Phenolic compounds are a kind of antioxidant agents which doing as free radical terminators and their bioactivities may be related to their abilities to chelate metals, prevent lipoxygenase and scavenge free radicals (Saha and Saxena, 2013).

Also the results of the inhibition radical scavenging assay by using DPPH shown in the table (7) by using ascorbic acid as standard.

Many radical kinds of various reactivity are formed through a lipid oxidation ($OH^{\cdot}$, $O_2^{\cdot -}$, $L^{\cdot}$, $LOO^{\cdot}$ $LO^{\cdot}$, etc.). Comparatively stable organic radical DPPH has been widely utilized in the determination of the antioxidant activity of single compounds as well as the various plant extracts (Kulisic *et al*., 2004). DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Hinneburg *et al*., 2006). The results shown as above the ethanolic extract has the largest percent of inhibition compared with other extracts and juice because the reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups. (Umamaheswari and Chatterjee, 2008).

Table 1. The qualitative analysis of *Actinidia deliciosa* extracts and juice

Phytochemical constituents	Ethanolic Extracts	Hot Extracts	Cold Extracts	Juice Kiwifruit	Color
Alkaloids	+++	++	+++	+++	Turbidity
Amino acids or primary and secondary amine	+++	++	++	+++	Blue - violet
Anthroquinones	-	-	-	-	-
Flavonoids	+++	+++	+	+	Yellow
Glycosides	+++	++	++	+++	Brown ring
Phlobatanins	-	-	-	-	-
Saponins	-	-	-	-	-
Tannins	-	-	-	-	-
Terpenoids	+++	+++	++	+++	Reddish brown

Where as :- (+):Present, (++): Mildly present, (+++): Strongly present, (-):Absent

Table 2. The quantitative analysis of phytochemical screening of *Actinidia deliciosa*

Phytochemical constituents	The percentage of products %			
	Alkaloids	Flavonoids	Saponins	Tannins
Ethanolic extract	4.47 %	7.61 %	-----	-----
Hot extract	2.92 %	5.03 %	-----	-----
Cold extract	3.15 %	5.63 %	-----	-----
Juice	3.98 %	6.685 %	-----	-----

Table 3. Malondialdehyde levels (µmoles /L) for *Actinidia deliciosa.* Extracts and juice

Sample	Malondialdehyde MDA (µmole /L)
Ethanolic extract	42.698
Hot extract	17.866
Cold extract	11.724
Juice	16.234

Table 4. The percent of Lipid peroxidation for *Actinidia deliciosa* (extracts and juice)

Sample	The percent of Lipid peroxidation
Ethanolic extract	70.3 %
Hot extract	63 %
Cold extract	57 %
Juice	49 %

Table 5. Vitamin E levels (mg / L) for *Actinidia deliciosa* extracts and juice

Sample	**Vitamin** E **(**mg/L**)**
Ethanolic extract	4.98
Hot extract	3.08
Cold extract	2.61
Juice	2.36

Table 6. The Total Phenolic Contant of *Actinidia deliciosa* extracts and juice (n=3)

Sample	Total phenolic content mg/g ± S.D
Ethanolic extract	187.91 ± 0.0508
Hot extract	184.52 ± 0.0202
Cold extract	184.08 ± 0.0236
Juice	183.82 ± 0.0405

Table 7. The Inhibition percentage of DPPH for *Actinidia deliciosa* extracts and juice (n=3)

Sample	Inhibition of DPPH %	Concentration of ascorbic acid mg/ml ± S.D
Ethanolic extract	72.5%	188.00 ± 0.0217
Hot extract	65.25%	180.75 ± 0.0561
Cold extract	61.00%	176.5 ± 0.0537
Juice	70.25%	185.75± 0.0367

Figure 4. Qualitative analysis of Phytochemical constituents for *Actinidia deliciosa* (extracts, Juice)

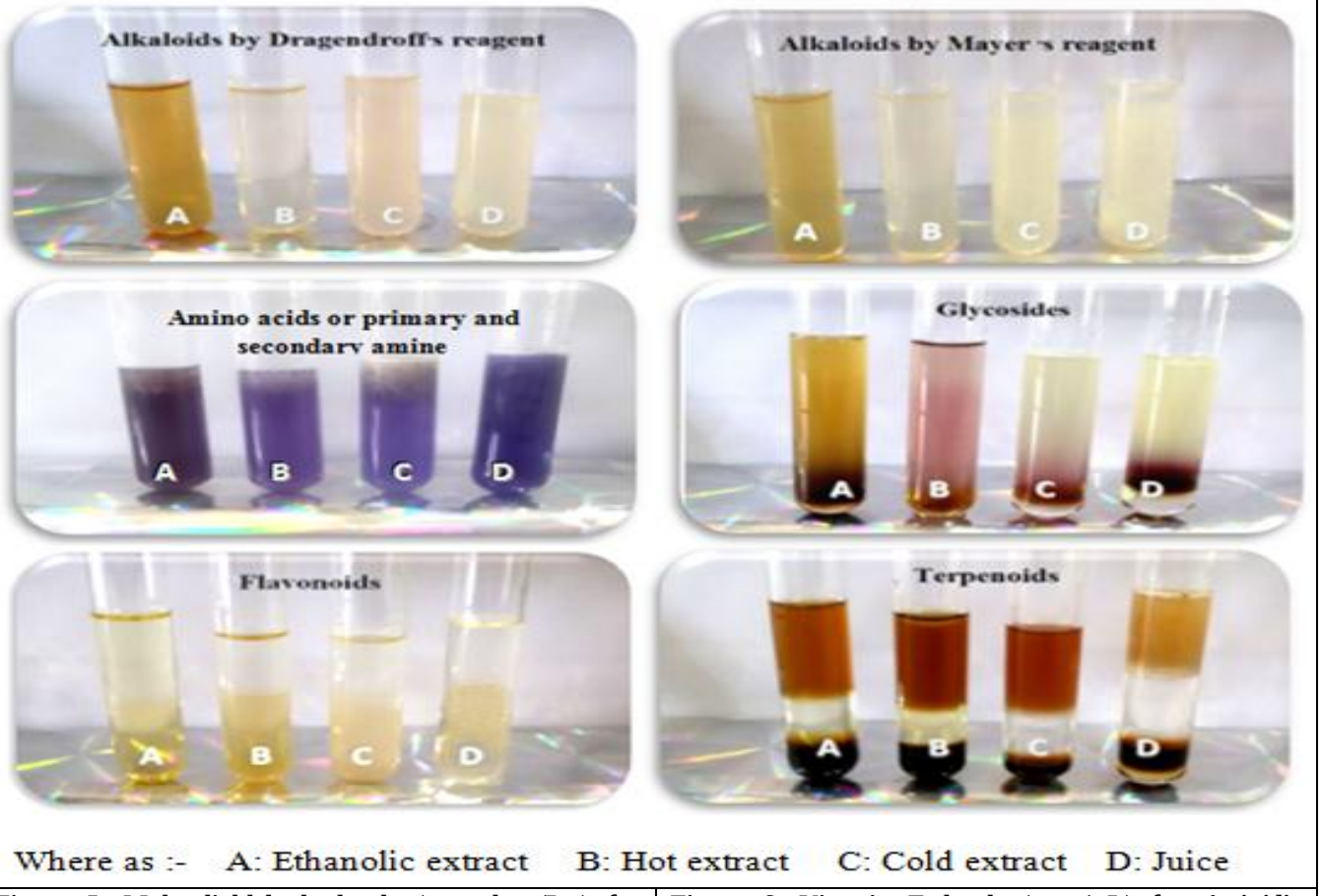


Figure 5. Malondialdehyde levels (µmoles /L) for *Actinidia deliciosa* extracts and juice

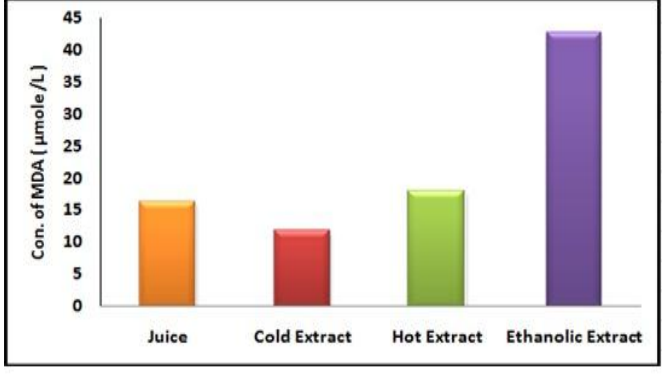
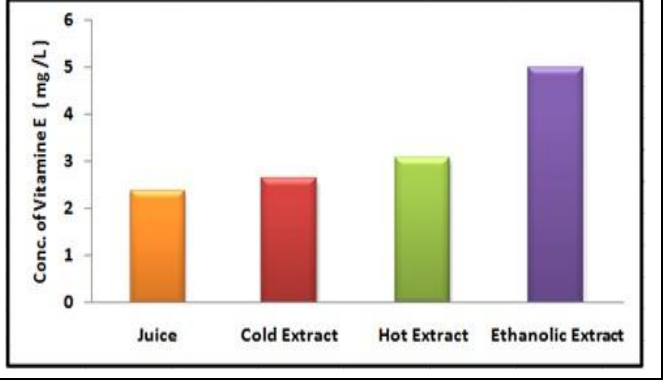


Figure 6. Vitamin E levels (mg / L) for *Actinidia Deliciosa* extracts and juice



CONCLUSIONS

The conclusions of this study indicate that *Actinidia deliciosa*. extracts and juice have rich phytochemical species via qualitative and quantitative. Ethanolic extract is better than aqueous cold extract, hot extract and juice because it may isolate most of active components that act as antioxidants.

ACKNOWLEDGEMENT: None

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

REFERENCES

Abd EIslam NM, Ullah R and Waseem M, Phytochemical analysis of medicinal plants Ranunculus arvensis. *Life Science Journal*, 10 (7s), 2013, 963 – 965.

Adamu HM, Ushie OA and Gwangwala AH, Estimation of Total Flavonoids and Tannins in the Stem Bark and Leaves of Anogeisus leiocarpus Plant. *Int J Trad Nat Med*, 2(3), 2013, 141-148.

Ali S, Salman SM, Jan MT, Afridi MS and Malik MS, Comparative Studies of Various Phytonutrients in Citrus Fruits. *Pak J Chem*, 4(2), 2014, 72-76.

Aliyu AB, Musa AM, Oshanimi JA, Ibrahim HA and Oyewale AO, Phytochemical Analyses and Mineral Elements Composition of some Medicinal Plants of Northern Nigerin. *Nig Journ Pharm Sci*, 7(1), 2008, 119- 125.

Almey AAA, Khan CAJ, Zahir I S, Suleiman K M, Aisyah M R and Rahim K K, Total phenolic content and primary antioxidant activity of methanolic and ethanolic extracts of aromatic plant's leaves. International Food Research Journal, 17, 2010, 1077-1084.Anand, sudhir K and Dutta MD,Vitamin E and its Role in the prevention of Atherosclerosis and carcinogenesis A Review. Journal of American college of Nutrition ,22(4), 2003, 258- 268.

Al-Temimi SSM and Al-Mashhedy LAM, Estimation of the Phytochemical Constituents and Biological Activity of Iraqi Ocimum sanctum L Extracts. *Int J Pharm Bio Sci*, 6 (1), 2015, 999-1007.

Anowi CF, Utoh-Nedosa A, Onyegbule A and Eze CC, Preliminary Phytochemical Studies and Evaluation of Antimicrobial Property of the Methanol Extract of the Roobark of Ritchiea longipedicellata Gilg Family Capparidacaea Wudpecker. *Journal of Pharmacognosy and Phytochemistry*, 1(6), 2013, 6-13.

Asadujjaman M, Hossain MA, and karmakar UK, Assessment of DPPH free radical scavenging activity of some medicinal plants. *Pharmacology On Line*, 1, 2013, 161 – 165.

Badmus JA, Adedosu TO, Fatoki JO, Adegbite V A, Adaramoye OA, and Odunola OA, Lipid Peroxidation inhibition and Antiradical Activities of some Leaf Fraction of Mangifera Indica. *Acta Poloniae Pharmaceutica - Drug Research*, 68 (1), 2011, 23 29.

Brigelius-Flohe B and Maret G, Vitamin E function and metabolism. *The FASEB J*, 13,1999, 1145-1152.

Bursal E and Gülçin İ, Polyphenol contents and in vitro antioxidant activities of lyophilised aqueous extract of kiwifruit Actinidia deliciosa. *Food Research International*, 44, 2011, 1482–1489.

Chinedu SN,Oluwadamisi AY, Popoola ST, David BJ and Epelle T, Analyses of the Leaf,Fruit and Seed of Thaumatococcus daniellii Benth Exploring Potential Uses. *Pakistan Journal of Biological Sciences*, 17(6), 2014, 849-854.

Dhongade H J and Chandewar A V, Pharmacognostical and Phytochemical studies of Phyllanthus amarus leaves. *International Journal of Biomedical And Advance Research*, 4(6), 2013, 382-389.

Edeoga HO, Okwu DE and Mbaebie BO, Phytochemical constituents of some Nigerian Medicinal plants. *African Journal of Biotechnology*, 4(7), 2005, 685-688.

Ghumare P, Jirekar DB, Farooqui M and Naikwade SD, Phytochemical analysis of petroleum ether extract of some selected medicinal plants leaves. *Indian Journal of Advances in Plant Research*, 1(5), 2014, 20-23.

Guidet B and Shah S, Enhanced in vivo H_2O_2 generation by rat kidney in glycerol – induced renal failure. *A M J Phsio*, 1257, 1989, 440 – 444.

Halimoon N and Abdul Hasan M H, Determination and Evaluation of Antioxidative Activity in Red Dragon Fruit *Hylocereus undatus* and Green Kiwi Fruit *Actinidia deliciosa*. *American Journal of Applied Sciences*, 7 (11), 2010, 1432-1438

Hashim S A and Schuttringer G R, Rapid determination of tocopherol in macro-and micro quantities of plasma results obtained in various nutrition and metabolic studies. *AmJ ClinNutr*, 19 (2), 1966,137-145.

Hinneburg I, Dorman HJD and Hiltunen R, Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chemistry*, 97, 2006, 122–129.

Huang S, Ding J, Deng D, Tang W, Sun H, Liu D, Zhang L, Niu X, Zhang X, Meng M, Yu J, Liu J, Han Y, Shi W, Zhang D, Cao S, Wei Z, Cui Y, Xia Y, Zeng H, Bao K, Lin L, Min Y, Zhang H, Miao M, Tang X, Zhu Y, Sui Y,Li G, Sun H, Yue J, Sun J, Liu F, Zhou L, Lei L, Zheng X, Liu M, Huang L, Song J, Xu C, Li J, Ye K, Zhong S, Lu B -R, He G,

Xiao F, Wang H – L, Zheng H, Fei Z and Liu Y, Draft genome of the kiwifruit Actinidia chinensis. *Nature Communications*, 4, 2013, 1-9.

Javanmardi J, Stushnoff C, Locke E and Vivanco JM, Antioxidant activity and total phenolic content of Iranian *Ocimum accessions*. *Food Chemistry*, 83, 2003, 547-550.

Khalili R M A, Shafekh S E, Norhayati AH, Fatahudin I M, Rahimah R, Norkamaliah H and Azimah A N, Total Phenolic Content and In vitro Antioxidant Activity of Winged Bean Psophocarpus tetragonolobus. *Pakistan Journal of Nutrition*, 12(5), 2013, 416-422.

Krishnaiah D, Devi T, Bono A, and Sarbatly R, Studies on phytochemical constituents of six Malaysian medicinal plants. *Journal of Medicinal Plants Research*, 32, 2009, 067-072.

Kulisic T, Radonic A, Katalinic V and Milos M, Analytical, Nutritional and Clinical Methods Use of deferent methods for testing antioxidative activity of oreganoessential oil. *Food Chemistry*, 85, 2004, 633-640.

Lawrence GHM, Taxonomy of Vascular plants, The macmilian company publisher, New York, p599-600, 1951.

Majaw S and Moirangthem J, Qualitative and Quantitative Analysis of Clerodendron colebrookianum Walp Leaves and Zingiber cassumunar Roxb Rhizomes. *Ethnobotanical Leaflets*, 13, 2009, 578-589.

Mir MA, Sawhney SS and Jassal MMS, Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale. *Journal of Pharmacy and Pharmacology*, 2(1), 2013, 001 – 005.

Nagib RM, Combined Effects of Methionine and Kiwi Fruit on Paracetamol Induced Liver Injury. *World Journal of Medical Sciences*, 9(1), 2013, 01-07.

Njoku O V and Obi C, Phytochemical constituents of some selected medicinal plants. *African Journal of Pure and Applied Chemistry*. 3 (11), 2009, 228-233.

Olajide J A, Danladi N B and Maria A S, In vitro antibacterial efficacy of crude ethanolic leaf extract and alkaloidal fractions of Phyllanthus Amaruson uro-pathogens. *Cent Euro J Exp Bio*, 1(2), 2012, 53-61.

Pandey N, Chaurasia JK,Tiwari OP and Tripathi YB, Antioxidant properties of different fractions of tubers from Pueraria tuberosa Linn. *Food Chemistry*, 105, 2007, 219–222.

Park YS, Im MH, Ham K-S, Kang S-G, Park Y-K, Namiesnik J, Leontowicz H, Leontowicz M, Katrich E and Gorinstein S, Nutritional and Pharmaceutical Properties of Bioactive Compounds in Organic and Conventional Growing Kiwifruit. *Plant Foods Hum Nutr*, 68, 2013, 57- 64.

Peter J, Backialakshmi M, Karpagavinayagam P and Vedhi C, Green Synthesis and Characterization of Colloidal Gold Nanoparticles for Optical Properties, *Journal of Advanced Chemical Sciences*,1, 2015, 1-5.

Poorna CA, Resmi MS and Soniya EV, In Vitro Antioxidant Analysis and the DNA Damage Protective Activity of Leaf Extract of the Excoecaria Agallocha Linn Mangrove Plant. *Int J Chem*,1(4), 2012, 1-6.

Prasad M P, Jayalakshmi K and Rindhe GG, Antibacterial activity of Ocimum species and their phytochemical and antioxidant potential. *International Journal of Microbiology Research*,4(8), 2012, 302-307.

Saha R and Saxena J, Screening of Total Phenolic and Flavonoid Content in Conventional and Non-Conventional Species of Curcuma. *Journal of Pharmacognosy and Phytochemistry*, 2(1), 2013, 176-179.

Sharma V K, Kumar S, Patel H J and Hugar S, Hypoglycemic Activity of Ficus Glomerata in Aloxan Induced Diabetic Rats. *International Journal of Pharmaceutical Science Review and Research*, 1(2), 2010,18-22.

Sharma V, Pracheta, Paliwal R and Sharma S, In vitro free radical scavenging and antioxidant potential of ethanolic extract of euphorbia Neriifolia Linn. *International Journal of pharmacy and pharmaceutical sciences*, 3(1), 2011, 238-242.

Shehata MSM, Soltan SSA, Effects of Bioactive Component of Kiwi Fruit and Avocado Fruit and Seed on Hypercholesterolemic Rats. *World Journal of Dairy & Food Sciences*, 8(1), 2013, 82-93.

Singh AR, Bajaj VK, Sekhawat P S and Singh K, Phytochemical estimation and Antimicrobial activity of Aqueous and Methanolic extract of *Ocimum Sanctum* L. Journal of Natural Product and Plant Resource, 3(1), 2013,51-58.

Sivapalan SR, Phytochemical study on medicinal plant – Sida cordifolia Linn. *International Journal of Mult idisciplinary Research and Development*, 2(1), 2015, 216-220.

Sochor J, Ruttkay-Nedecky B, Babula P, Adam V, Hubalek J, and Kizek R, Automation of Methods for Determination of Lipid Peroxidation, InTech Publisher,Chapter 6, 2012.

Soni H and Singhai A K, A Recent Update of Botanicals for Wound Healing Activity. *International Research Journal of Pharmacy*, 3(7), 2012, 1-7.

Stanković MS, Total Phenolic Contents, Flavonoid concentration and Antioxidant Activity of Marrubium peregrinum L Extracts. *Kragujevac J Sci*, 33, 2011, 63-72.

Thirunavukkarasu P, Ramanathan T, Shanmugapriya R, Umamaheswari G and Renugadevi G, Antioxidant and Free Radical Scavenging Effect of A canthus ilicifolius. *Res J Applied Sci*, 6(3), 2011, 218 – 222.

Raghuvanshi R, Kaul A, Bhakuni P, Mishra A and Misra M K, Xanthine Oxidase as amarker of Myocardial Infarction. *Indian Journl of Clinical Biochemistry*, 22(2), 2007, 90-92.

Rashid F, Sharif N, Ali I, Sharif S, Un Nisa F and Naz S, Phytochemical analysis and inhibitory activity of Ornamental Plant Bougainvillea spectabilis. *Asian J Plant Sci Res*, 3(2), 2013,1-5.

Rukmini K, Devi P S, Chitturi C M K, Gc-Ms Analysis and Phytochemical Screening of a Fern Hemionits Arifolia BURM Moore from Tirumala Hills. *Int J Pharm Bio Sci*, 6(1), 2015, 1360 – 1369.

Uma C and Sekar KG, Phytochemical analysis of a folklore medicinal plant citrullus colocynthis L bitter apple. *Journal of Pharmacognosy and Phytochemistry*, 2(6), 2014, 195-202.

Umamaheswari M and Chatterjee T K, In Vitro Antioxidant Activites of the Fractions of Coccinia Grandis L Leaf extract, Afr J Traditional ,*Complementary and Alternative Medicines*,5 (1), 2008, 61 – 73.

Vaishali RM, Pai VR, Pratapchandra HK and Hegde S, Preliminary Phytochemical Screening of Members of Lamiaceae Family Leucas linifolia, Coleus aromaticus and Pogestemon patchouli. *International Journal of Pharmaceutical Sciences Review and Research*, 21(1), 2013, 131-137.

Vinotha S, Thabrew I and Ranjani SS, Preliminary Phytochemical Screening of Different Extracts of Whole Plant of Enicostemma littorale Blume. *International Journal of Sciences Basic and Applied Research*,11(1), 2013, 99-104.

Wadood A, Ghufran M, Jamal S B, Naeem M, Khan A, Ghaffar R and Asnad, Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. *Biochemistry & Analytical Biochemistry*, 2(4), 2013, 1- 4.

Wang Y S, Ding M D, Gu X G, Wang J L, Pang Y, Gao L P and Xia T, Analysis of Interfering Substances in the Measurement Malondialdehyde Content in Plant Leaves, *American Journal of Biochemistry and Biotechnology*, 9(3), 2013, 235-242.

Wankhade D M, Investigation on callus formation in Piper nigrum L. *International journal of pharmaceutical and chemical sciences*, 3(2), 2014, 358-366.

Yang H, Lee Y-C, Han K-S, Singh H, Yoon M, Park J-H, Cho C-W and Cho S, Green and gold kiwifruit peel ethanol extracts potentiate pentobarbital-induced sleep in mice via a GABAergic mechanism. *Food Chemistry*, 136, 2013, 160–163.

Zahin M, Aqil F and Ahmad I, The in vitro antioxidant activity and total phenolic content of four Indian medicinal plants. *International Journal of Pharmacy and Pharmaceutical Sciences*, 1 (l), 2009, 1-8.

Zendehbad S H, Mehran M J and Malla S, Flavonoids and Phenolic content in wheat grass plant Triticum Aestivum. *Asian J Pharm Clin Res*, 7(4), 2014, 184-187.

Zohra SF, Meriem B, Samira S and Muneer A, Phytochemical Screening and identification of some compounds from Mallow. *Journal of Natural Product and Plant Resourse*. 2(4), 2012, 512-516.