International Journal Water Resources Management and Irrigation Engineering Research

Vol 2, No.1, pp.1-10, September 2019

Published by European Centre for Research Training and Development UK (www.eajournals.org)

PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT PROPERTIES OF METHANOLIC LEAF EXTRACT OF *PUNICA GRANATUM L.*

Bintu Babagana, Binta Baba Shehu, Aliyu Daja and Madu Adamu Gadaka.
Department of Biochemistry, Faculty of Science, University of Maiduguri,
P. M. B. 1069, Maiduguri, Nigeria.

ABSTRACT: *Phytochemical constituent and antioxidant properties of the methanolic leaf extract of Punica granatum was investigated. Extracts of the leaf was subjected to quantitative and qualitative phytochemical screening, In vivo and In vitro scavenging activity against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and hydrogen peroxide (H_2O_2) were determined. The free radical scavenging activity of DPPH and H_2O_2 have SC_{50} of 1.069±0.29µmol/ml and 1.819±0.29µmol/ml respectively. DPPH actitvity of these extract shows no significant difference when compared with a standard value of Vitamin C is 1.140±0.26µmol/ml whereas H_2O_2 shows significant difference as compared to standared Vitamin C with SC_{50} value of 1.331±0.31µmol/ml. The administration of the extract at 200mg/kg and 400mg/kg body weights of wistar albino rats significantly increased the levels of catalase 39.02±0.72nmol/mg and 39.55±0.62nmol/mg respectively with a negative control of 7.53±1.94nmol/mg as compared to the normal control value of 37.76±0.63nmol/mg whereas lipid peroxidation level decreased significantly at treatment groups 200mg/kg and 400mg/kg with value of 4.96±0.20(nmol/mg)/10^{-6} and 4.35±0.17(nmol/mg)/10^{-6} respectively when compared to the negative control group with a value of 8.21±0.15(nmol/mg)/10^{-6} and normal control value of 4.36±1.67(nmol/mg)/10^{-6}, although there is no significant difference as compared to the normal control groups. These findings suggest possible antioxidant properties of the methanolic leaf extract of Pomegranate.*

KEY WORDS: *Punica granatum*, free radicals scavenging activity, oxidative stress, CCL_4, DPPH, antioxidant activity.

INTRODUCTION

The Pomegranate, botanical name '*Punica granatum',* is a fruit bearing deciduous shrub that contains thousands of health benefits as mentioned three times in the holy Qur'an, thus showing its advantage and privilages as fruits that are beneficial to human beings. In English, it is known as the 'Pomegranate' and '*al- Rumman'* or '*al- Rummanah'* in Arab.(Munirah, 2011.) in older times, the fruit considered in old testament of the Bible, the Jewish Torah, and the Babylonian Talmud as a sacred fruit conferring powers of fertility, abundance, and good luck.(Akhlaghi and Band, 2009.)

Pomegranate or Punica granatum, is a small tree growing between 5 and 8 meters (16-26ft) tall, leaves are glossy and lance shaped, it is considered to have originated from the area of southwest Asia and has grown naturally in the entire Mediterranean region and the Caucasus since ancient time, it can be divided into several anatomical compartments including seed, juice, peel, leaf,

flower, bark and root with each possessing interesting pharmacological and toxicological activities. (Tanner and Cory, 2009.).

Punica granatum L. is a nutrient dense fruit rich in phytochemical compounds, as well as strong antioxidant and antiinflamatory properties. (Miguel *et.al.,* 2010). Recent studies have demonstrated its anti-cancer activity in several human cancers (Adhami and Mukhtar, 2007; Longtin, 2003). Apart from their antioxidant capacity, there have been numerous reports on the in vivo properties of pomegranates, namely on anti-atherosclerotic capacity (Aviram and Dornfeld, 2001; Kaplan and Aviram, 2001), anti-proliferative and pro-apoptotic activities of pomegranate tannin extract (Seeram *et al*., 2005), antiinflammatory activity (Adams *et al*., 2006), as well as chemopreventive and chemotherapeutical potential towards prostate cancer by pomegranate juice (Malik et al., 2005).

The pomegranate contains several major active components including flavonoids.(Jurenka, 2008; Marhari *et.al.*,2014; Suranto and Terbukti, 2011; Arun and Singh, 2012; Yasoubi, 2007.). The benefits of flavonoids are as antibacterial, antiviral, anti-insecticide and anti-inflammatory while the tannin substances as hemostatic, antibacterial, antioxidant and anti-inflammatory.(Prashanth *et.al.*,2001).

The role of antioxidants in human health has prompted some studies in the fields of food science and horticulture to assess fruit and vegetable antioxidants (Kalt *et al*., 1999). They act by scavenging free radicals and by terminating any free radical-induced chain reaction. When an animal is exposed to an external source of free radicals (e.g., as a result of exposure to a toxic chemical) the animals antioxidant defense mechanism steps in and tries to control the rate of deleterious bio-oxidation and molecular damage. The DPPH and H_2O_2 radicals were widely used to investigate the scavenging activity of some natural compounds.

Jeon *et al*., 2003 reported that; Carbon tetrachloride (CCl_4) is an extensively studied xenobiotic that induces lipid peroxidation and toxicity. Carbon tetrachloride (CCl_4) induced Liver cell injury by involves initially the metabolism of CCl_4 to trichloromethyl (CCl_3) free radical by the mixed function oxidase system of the endoplasmic reticulum. The secondary mechanism could involve the generation of toxic products arising directly from CCl_4 metabolism or from peroxidative degeneration of membrane phospholipids, and causes functional and morphological changes in the cell membrane leading to accumulation of lipid-derived oxidants causing liver injury. Moreover, reactive oxygen intermediates (ROIs) generated in the hepatocytes as by-products of CCl_4 metabolism and excess of ROIs, oxidative stress, contribute to cell injury (Kumaravelu *et al*., 1995). CCl_4 induced damage also produces alteration in the antioxidant status of the tissues, which is manifested by abnormal histopathological changes. Several studies have previously demonstrated that antioxidants prevent CCl_4 toxicity, particularly hepatotoxicty, by inhibiting lipid peroxidation and increasing antioxidant enzyme activities (Kumaravelu *et al*., 1995). Different parts of *Punica granatum L.* were scientifically tested for their medicinal properties by previous workers worldwide, but little information is available on the leaves particularly in Nigeria. This paper therefore determines the Phytochemical constituents and antioxidant properties of Methanolic leaf extract of *Punica Granatum L*.

MATERIALS AND METHODS

Chemicals

Reagents and chemicals used for the experiment are of analar grades. DPPH and Folin Ciocalteau were bought from Sigma Aldrich, United States. All other reagents and chemicals were purchased from various manufacturers.

Plant collection and identification

The fresh leaves of pomegranate were collected from Botanical garden, University of Maiduguri, Borno State, Nigeria and was identified by a plant taxonomist at the Department of Biological Sciences, University of Maiduguri, Nigeria.

Preparation of plant materials

Following collection, the fresh leaves of *Punica granatum Linn* was washed and shade dried. It was then ground into fine powder using mortar and pestle. It was then sieved to remove debris and coarse plant materials. A powder form of the leaves was stored under laboratory condition prior to extraction.

Methanolic extract preparation

About 500g powder of *Punica granatum Linn* leaf was extracted with one liter of 70% methanol using Soxhlet extractor, the extract was then concentrated to dryness at low temperature on a rotary evaporator. The percentage yield of the extract was 13.54%.

Phytochemical screening of Pomegranta methanolic leaf extract (PMLE)

The pomegranate methanol leaf extract (PMLE) was subjected to preliminary phytochemical (qualitative and quantitative) screening/analyses for identification of the various classes of the plant chemical constituents. These analyses were carried out according to the methods described by (Sudiarto and Rifai, 1992).

Phytochemical screening/Analysis of leaf of *Punica granatum*

Phytochemical analysis of the *Punica granatum* leaf was determined using standard analytical methods. Tannins and Flavonoids were determined by the method of Krishnaiah (2009), Alkaloids by Harbone (1998), phenolics by the method of Antolovich (2002) and Ascorbic acid by method as described by Kirk and Sawyer (1998).

The qualitative analysis of *Punica granatum* leaf was determined by the standard method as described by Trease and Evans (2002).

In vitro and *In vivo* Antioxidant activity study

DPPH free radical scavenging activity was determined by the method of Yen and Duh (1994), Hydrogen peroxide scavenging (H_2O_2) assay was as described by (Ruch *et al*., 1989). The *In vivo* antioxidant activity was determined by method of Draper and Hadley (1990), determination of lipid and catalase activity was done as described by Atawodi (2007).

RESULTS

Phytochemical Constituents

Qualitative Phytochemical Analysis of methanolic leaf extract of *Punica granatum L.*

The result of qualitative phytochemical analysis (Table 1) revealed the presence of alkaloids, flavonoids, saponins, carbohydrates, phenol, steroids, and tannins but lacked terpenoids.

Table 1: Qualitative Phytochemical Composition of Methanolic Leaf Extract of *Punica granatum.*

Test	Result
Carbohydrate	+
Tannins	+
Flavanoids	+
Alkaloids	+
Steroids	+
Saponin	+
Terpenoids	-
Phenol	+

Key: + Presence,
- Absent

Quantitative Phytochemical Analysis methanolic leaf extract of *Punica granatum L.*

The result of quantitative phytochemical analysis of the *Punica granatum L*. leaf extract is presented on Table 2. Concentration of phenol 3.70 mg/GAE, Alkaloids 2.03 g, Flavanoids 0.794 g, Tannins 0.616 g and Ascorbic acid VitCmg=1261.3 mg/100g. All these are shown in Table 2.

Table 2: Qualitative Phytochemical Composition of Methanolic Leaf Extract of *Punica granatum.*

Phytochemicals	Concentration (g)
Tannin (g)	0.61 ± 0.01
Flavanoid (g)	0.79 ± 0.01
Alkaloid (g)	2.05 ± 0.01
Phenol (mg/GAE)	3.70 ± 0.17

Values are presented as mean ± SEM,
GAE; Garlic acid equivalent.

Free radical scavenging activity of the methanolic leaf extract of *Punica granatum L.*

The result of this study showed different levels of DPPH free radical scavenging activity with SC_{50} of 1.069±0.29mg/ml as compared to the standard Ascorbic acid 1.140±0.26mg/ml which has no significant difference at $P < 0.05$, and that of Hydrogen Peroxide scavenging activity which is significantly different with SC_{50} of 1.819±0.29mg/ml as compared to the standard 1.331±0.31mg/ml. (Table 3).

Table 3: Free radical scavenging activity (SC_{50}) in µmol/l of methanolic leaf extract of *P.granatum*.

Antioxidant	SC_{50} (DPPH)	SC_{50} (H_2O_2)
Punica granatum (µmol/ml)	1.069±0.29	1.819±0.29*
Vitamin C (µmol/ml)	1.140±0.26	1.331±0.31

Values are presented as mean ± SEM, of four determination.
Values with different asteric (*) vertically down the column are significantly different. ($P < 0.05$).

Effect of the Methanolic Leaf Extract of *Punica granatum* on *In vivo* Antioxidant parameter (Catalase and Lipid peroxidation).

The Results in Table 4 reveal that Carbon tetrachloride caused a significant reduction in the level of catalase in the negative control group as compared to treatment groups where the plant extract caused a significant increase in the levels while lipid peroxidation level was significantly increased with CCL_4 administration as extract administration significantly decreased the levels close to that of the normal control group.

Table 4: Effect of the Methanolic Leaf Extract of *Punica granatum* on *In vivo* Antioxidant parameter (Catalase and Lipid peroxidation) after 28 days of extract administration.

Group	Catalase (nmol/mg)	Lipid peroxidation (nmol/mg)/10^{-6}
Normal control group	37.76 ± 0.63^{a}	4.36 ± 1.67^{a}
Negative control group (CCL_4)	7.53 ± 1.94^{b}	8.21 ± 0.15^{b}
Positive control group (drug silymarin)	43.72 ± 0.73^{c}	5.88 ± 0.37^{a}
Treatment group (200mg/kg)	39.02 ± 0.72^{a}	4.96 ± 0.20^{a}
Treatment group (400mg/kg)	39.55 ± 0.62^{a}	4.35 ± 0.17^{a}
Extract group (200mg/kg)	45.30 ± 0.51^{dc}	3.96 ± 1.61^{a}
Extract group (400mg/kg)	43.85 ± 1.61^{ec}	5.72 ± 4.15^{a}
Extract group (800mg/kg)	36.31 ± 2.77^{fd}	3.97 ± 1.93^{a}

Values are presented as mean ± SEM, n = 5
Values with different superscript vertically down the group are significantly different ($p < 0.05$)

DISCUSSION

Preliminary phytochemical screening carried out in this study indicated that *Punica granatum* leaf extract contained alkaloids, flavonoids, saponins, carbohydrates, steroids, phenols and tannins, but lacked terpenoids. Pomegranate is an important source of anthocyanins, hydrolysable tannins punicalagin and punicalin (Afaq *et al.*, 2005), ellagic and gallic acids (Lansky and Newman, 2007) and also contains vitamin C (Turk *et al.*, 2008). These phytochemicals are known to perform several general and specific functions in plants, and may exhibit different biochemical and pharmacological actions in different species of animals when ingested. These actions range from cell toxicity to cell protective effects (Trease and Evans,

1996). The quantitative phytochemical analysis of the methanolic leaf extract of *Punica granatum* Linn. showed that concentrations of alkaloids, flavonoids, tannins and phenols, which are known to exhibit pharmacological activities are high. Flavonoids are potent water soluble antioxidants and free radical scavengers that prevent oxidative cell damage, have strong anticancer activity and protect against the different levels of carcinogenesis (Okwu, 2004). The accumulation of these compounds in plant cells have attracted the attention of scientists worldwide and have been the subject of intense investigations. Tannins also react with proteins to produce the typical tannins effect which is important in the treatment of inflammed or ulcerated tissues, burns, wounds, pneumonia and dysentery (Mortan *et al.,* 1987). Plant parts that contain tannins are astringent in nature and have important roles as stable and potent antioxidants (Dharmanda, 2003). Flavonoids have also been found to play a very important role in protection against oxidative stress (Said *et al*., 2008). As shown in Table 2, flavonoids, tannins and alkaloids were found in high concentrations. Thus the high tannins, alkaloids and flavonoids contents of this plant may explain their therapeutic use in herbal medicine**.**

The methanolic leaf extract of *Punica granatum* was found to exert significant antioxidant effect in DPPH and H_2O_2 radical scavenging assay with SC_{50} values of 1.069±0.29µmol/ml and 1.819±0.29µmol/ml respectively as compared to the standard Ascorbic acid (1.140±0.26µmol/ml and 1.331±0.31µmol/ml respectively), this can be related to the work conducted by (Ahmed *et al*., 2011), it was observed that Pomegranate juice (PJ) and Methanolic extract of Pomegranate Pulp (MEPP) had significant effect on some oxidants/antioxidants enzymes of liver and kidney when compared to the control. The therapeutic potentials of plants have been linked with their antioxidant potentials (Akinmalodun *et al*., 2007; Eleazu *et al*., 2011). The decrease in absorbance of DPPH and H_2O_2 caused by the methanolic leaf extract of *Punica granatum* is due to the reaction between antioxidant molecules and radicals, which results in the scavenging of the radical by hydrogen donation.

The *In vivo* antioxidant studies (catalase and Lipid peroxidation) revealed a decreased value of 7.53± 1.94 in the negative control group as compared to normal control (37.76± 0.63). The administration of the extract significantly increased the levels to a level similar to that of the normal control group at two different doses of 200mg and 400mg per kg body weights with values of 39.02±0.72 and 39.55±0.62 respectively for catalase. This shows that the methanolic leaf extract of *Punica granatum* has a significant effect on the catalase activity. There is also a significant increase in the lipid peroxidation with a value of 8.21±0.15 in the negative control group as compared to the normal control (4.36±1.67), administration of the extract therefore decreased the levels to a level closer to that of the normal control values at two different doses of 200mg and 400mg per kg body weights with values of 4.96±0.20 and 4.35±0.17 respectively at $p<0.05$. Several researchers have observed that the activities and expression of antioxidant enzymes such as catalase, glutathione peroxidase and SOD drops drastically in the liver and kidney of animals exposed to an external source of free radicals compared to control animals (Khalaf *et al.,* 2009, Hamed *et al.,* 2016, Mahmoud *et al.,* 2015, Hafez *et al.,* 2014, Zhang *et al.,* 2013, El-Nekeety *et al.,* 2014). This may be explained by the fact that these radicals may inhibit (non-specifically) the synthesis as well as the activity of antioxidant enzymes in the liver and

kidney at the DNA level and at the protein level respectively (Birben *et al.,* 2012, Weber *et al.,* 2003, Dalton *et al.,* 1999, Siu and Draper, 1982).

CONCLUSIONS

Punica granatum methanolic leaf extract has antioxidant property and hence it may be used for therapeutic purposes.

Recommendation

The mechanism of action as well as isolation of compound responsible for this effects should be studied.

References

Adams L. S, Seeram N. P, Aggarwal B. B, Takada Y, Sand D, and Heber D. (2006). Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. *Journal of Agricultural Food and Chemistry*, 54(3), 980-985.

Adhami V. M, and Mukhtar H. (2007). Anti-oxidants from green tea and pomegranate for chemoprevention of prostate cancer. *Mol. Biotechnol*., 37: 52-57.

Afaq F, Saleem M, Krueger C. G, Reed J. D, and Mukhtar H. (2005). Anthocyanin and hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF-kappaB pathways and inhibits skin tumorigenesis in CD-1 mice. *International Journal of Cancer*, 113(3), 423-433.

Ahmed E, Abdel Moneim, Mohamed A, Akhil and Saleh Al-Quraishy. (2011). Studies on the effect of Pomegranate (*Punica granatum*) juice and peel on liver and kidney in adult male rats. *Journal of Medicinal plants research.* Vol 5(20), Pp, 5083-5088.

Akhlaghi M, and Band B (2009). Mechanisms of flavonoids protection against myocardial ischemia reperfusion injury. *Journal of Molecular cell Cardiol*; 46:309-317.

Akinmalodun A. C, Ibukun E. O, Afor E, Akirinlola B. L, Onibon T. R, Akinboboye A. O, Obuotor E. M, and Farombi E. O. (2007). Chemical Constituents and Antioxidant Activity of *Alstonia boonei*. *Africa Journal of Biotechnology*, **6**(10): 1197- 1201

Antolovich M, Prenzler P. D, Patsalides E, M. C, Donalds S, and Robards K. (2002). Methods for testing antioxidant activity. *Analyst* 127: 183-198.

Arun N, and Singh D. P. (2012). *Punica granatum*: a review on pharmacological and therapeutic properties. *International Journal of Pharmaceutical Science and Res*; 3: 1240–1245.

Atawodi S. E. (2007). "Evaluation of the hypoglycemic, hypolipidemic and antioxidant effects of methanolic extract of "Ata-Ofa" polyherbal tea (A-polyherbal) in alloxan-induced diabetic rats," *Drug invention Today*, vol. 80, no.1, Pp, 44-84.

Aviram M. and Dornfeld L. (2001). Pomegranate juice consumption inhibits serum angiotensin converting enzyme activity and reduces systolic blood pressure. Atherosclerosis, 158(1), 195-198.

Birben E, Sahiner U.M, Sackesen C, Erzurum S, and Kalayci O. (2012). Oxidative stress and antioxidant defense. WAOJ. 2012:9-19.

Dalton T. P, Shertzer H. G, and Puga A. (1999). Regulation of gene rxpression by reactive oxygen. *Ann Rev pharmacol Toxicol*. 39:67-101.

Draper H. H and Hadley M. (1990). "Malondialdehyde determination as index of lipid peroxidation dismutase," *Methods in Enzymology,* vol. 186, Pp. 421-431.

Eleazu C. O, Okafor P. N, Amajor J, Awa E, Ikpeama A.I, and Eleazu K. C. (2011). Chemical Composition, Antioxidant Activity, Functional Properties and Inhibitory Action of Unripe Plantain (*M. Paradisiacae*) Flour. *African Journal of Biotechnology*, 10(**74**): 16948 - 6952.

El-Nekeety A. A, Abdel-Azeim S. H, Hassan A. M, Hassan N. S, Aly S. E, and Abdel-Wahhab M. A. (2014). Quercetin inhibits the cytotoxicity and oxidative stress in liver of rats fed aflatoxin-contaminated diet. *Toxicol Rep*; 1(2014): 319-29.

Hamed S. S, Al-yahya N. A, El-Khadragy M. F, Al-Olayan E. M, Alajmi R. A, Hassan Z, Hassan S. B, and Abdel Moneim A. E. (2016). The protective properties of the strawberry (Fragaria ananassa) against carbon tetrachloride-induced hepatotoxicity in rats mediated by antioxidant genes expression effect. *Front in physiol*;7:325.

Hafez M. M, Al-shabanah O. A, Al-Harbi N. O, Al-Harbi M. M, Al- Rejaie A. A, Alsurayea S. M, and Sayed-Ahmed M. M. (2014). Association between paraxonases gene expression and oxidative stress in hepatotoxicity induced by CCL_4. *Oxid Med Cell longer. Doi* 10.1155/2014/893212.

Harbone J. B. (1998). Phytochemical Methods. *Chapman and Hall Ltd. London, UK.*, Pp:49-188

Jeon T. I, S. G. Hwang, N. G. Park, Y. R. Jung, S. I. Shin, S. D. Choi, and D. K. Park. (2003). Antioxidative effect of chitosan on chronic carbon tetrachloride induced hepatic injury in rats. *Toxicology* 187: 67-73.

Jurenka J. (2008). Therapeutic applications of pomegranate (punica granatum L.): a review. *Alt Medical Review*. 13: 128–131, 137–138, 141.

Kalt W, C. F, Forney C. F, Martin A, and Prior R. L. (1999). Antioxidant capacity, vitamin C, phenolics and anthocyanins after fresh storage of small fruits. *Journal of Agriculture and Food Chemistry*, 47: 4638-4644.

Kaplan M. and Aviram M. (2001). Retention of oxidized LDL by extracellular matrix proteoglycans leads to its uptake by macrophages: An alternative approach to study lipoproteins cellular uptake. *Arterioscler. Thromb. Vasc. Biol*., 21: 386-393.

Khalaf A. A. A, Mekawy M. E. M, Moawed M. S, and Ahmed A. M. (2009). Comperative study on the protective effect of some antioxidants against CCl_4 hepatotoxicity in rats. *Egypt J. Nat tox*; 6(1): 59-82.

Kirk R. S, and Sawyer R. (1998). Pearson's composition and analysis of foods. Longman Education Publisher LTD UK, pp. 707-798.

Krishnaiah D, Devi T, Bono A, and Sarbatly R. (2009). Studies on Phytochemical Constituents of six Malaysian Medicinal Plants. *Journal of Medicinal Plants. Res. 3(2): 67-72.*

Kumaravelu P. D. P, Dakshinamoorthy S, Subramaniam H, Devaraj and N. S. Devaraj. (1995). Effect of eugenol on drugmetabolizing enzymes of carbon tetrachloride-intoxicated rat liver. *Biochemical Pharmacology*. 49: 1703-1707.

Lansky E. P, Newman R. A. (2007). *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J. Ethnopharmacol., 109: 177-206.

Longtin R. (2003). The pomegranate: Nature's power fruit? *Journal of National. Cancer Institute.*, 95: 346-348.

Mahmoud M. A, Ahmed R. R, Soliman H. A, and Salah M. (2015). Ruta graveolens and its active constituent rutin protect against diehtylnitrosamine-induced nephrotoxicity through modulation of oxidative stress. JAPS; 5(10):016-21.

Malik A, Afaq F, Sarfaraz S, Adhami V. M, Syed D. N, and Mukhtar H. (2005). Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer. *Proc. National Academic of Science.* 102: 14813-14818.

Marhari O. Y, and Dewi K. K. (2014). Khasiat ajaib delima. 1st ed. Jakarta: Padi; p. 1–5, 23–24, 63.

Miguel M. G, M. A. Neves, and M. D. (2010). Antunes, "pomegranate (*Punica granatum L.*): a medicinal plant with myriad biological properties- a short review," *journal of medicinal plant Research,* vol. 4, no. 25, pp. 2836- 2847.

Morton J, and Miami F. L. (1987). Pomegranate. In: Fruits of warm climates. p. 352–355.

Munirah Abd Razzak. (2011). *Punica Granatum Bicara* Al-Qur'an Al-Hadith *Dan sains perubatan Modern*, Jurnal Al-Bayan, bil. 9 (1), Universiti Malaya: Jabatan Al-Qur'an dan Al-Hadith, Akademi Pengajian Islam.

Okwu D. E. (2004). Phytochemicals and Vitamin Content of Indigenous Species of South Eastern Nigeria. *Journal of Sustain Agriculture and Environment*., **6:** 30 - 34

Prashanth D, Asha M. K, and Amit A. (2001). Antibacterial activity of *Punica granatum*. Fitoterapia. 72: 171–173.

Ruch R. J, Cheng J, Klaunig J. E. (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 10 (1989,) p. 1003.

Said O, Fulder S, Kkali K. A, Zaizeh H, Kassis E, and Saad B. (2008). Maintaining a physiological blood glucose level with Glucose level, a combination of four anti- diabetes plants used in traditional Arab herbal medicine. *Evidence-Based Complementary Alternative Medicine*. **5**: 421 - 428.

Seeram N. P, Adams L. S, Henning S. M, Niu Y, Zhang Y, Nair M. G, and Heber D. (2005). In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *Journal of Nutritional Biochemistry*., 16: 360-367.

Sudiarto and Rifai M. A. (1992). Plant Resources of South East Asia. Prosea Foundation. Bogor.

Sui G. M, and Draper H. H. (1982). Metabolism of malondialdehyde *in vivo* and *in vitro*. Lipids. 17:349=55.

Suranto A. (2011). Terbukti pome tumpas penyakit. 1st ed. Jakarta: Pustaka Bunda; Pp. 1–15, 31–33.

Tanner and Cory. (2009). *"pomegranate".* Horticulture Extension Department, Clemson University, 21 june 2014.

Trease, G. E. and W.C. Evans. (1996). Phenols and Phenolic Glycosides, In: Trease and Evans Pharmacognosy and Biliere Tindall, London. Pp 832.

Trease G. E and Evans W. C. (2002). Textbook of pharmacognosy. 14th Edn. W. B. Saunders Co. Ltd, London, U. K., Pp 24 – 28.

Turk G, Sonmez M, Aydin M, Yuce A, Gur S, Yuksel M, Aksu E. H, and Aksoy H. (2008). Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. *Clin Nutr.*, 27: 289-296.

Weber L. W, Boll M, and Stamfil A. (2003). Heptotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a Toxicological Model. *Crit Rev Toxicol.* 33(2):150-36.

Yasoubi P. (2007). Total phenolic contents and antioxidant activity of pomegranate (punica granatum L.) peel extracts. *Journal of Agriculture Science and Technology*. 9: 35–42.

Yen G. C, and Duh P. D. (1994). Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. *Journal of Agricultural Food Chemistry*. 42: 629-632.

Zhang S, Lu B, Han X, Xu L, Qi Y, Yin L, Zhao Y, Liu K, and Peng J. (2013). Protection of the flavonoid fraction from Rosa Levigata michx fruit against Carbon tetrachloride-induced acute liver injury in mice. *Food Chemical Toxicology;* 2013(55):60-9.