Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(1): 37-40
Received: 08-11-2016
Accepted: 09-12-2016

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Estimation of some phytoconstituents and evaluation of antioxidant activity in *Aegle marmelos* leaves extract

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Abstract
In existing study we carried out a systematic record of quantitative estimation of phytoconstituents in ethanolic extracts of *Aegle marmelos*. Total phenolic content was found to be 40.836±0.001mg/g, flavonoid content was 66.480±0.005mg/g, tannin content was 60.267±0.002 mg/g and carbohydrate was 37.530±0.570mg/g. Antioxidant activity was determined by DPPH (1,1-Diphenyl-2-picryl hydrazyl) method and by Reducing power assay in methanol, ethanol and aqueous extracts for 800µg/ml concentration. Highest radical scavenging activity was found in aqueous extract with IC_{50} value equal to 125µg/ml, reducing power assay also confirmed the same results.

Keywords: *Aegle marmelos*, antioxidant activity, phytoconstituents, 1,1-diphyenyl-2-picryl hydrazyl

1. Introduction
The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances (phytoconstituents) that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds (Akindele and Adyeyemi, 2007) [1].

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. A variety of free radical scavenging antioxidants exists within the body which many of them are derived from dietary sources like fruits, vegetables and tea (Sauria, 2007) [19]**.** It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart diseases, ageing, diabetes mellitus, cancer, immunosuppressant, neurodegenerative diseases and others. The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. (Shyura, 2005) [23].

Antioxidants may guard against reactive oxygen species (ROS) toxicities by the prevention of ROS construction, by the disruption of ROS attack, by scavenging reactive metabolites and converting them to less reactive molecules or by enhancing the resistance of sensitive biological target to ROS attack (Sen, 1995) [22]. Free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are associated with many pathological conditions such as atherosclerosis, arthritis, ischemia, reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999; Cook and Samman 1996) [12, 9]. Synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroxy quinone and gallic acid esters have been suspected to be carcinogenic. Hence, strong limitations have been placed on their use and there is a trend to replace them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity (Barlow, 1990; Branen, 1975) [4, 7]. Hence, search for natural antioxidant has greatly been increased in the recent scenario. Many crude extracts and pure natural compounds have been reported which have potent antioxidant potential (Schuler, 1990; Chu, 2000; Mantle *et al*., 2000) [21, 14]. However there is still a need to find out more effective antioxidant having fewer side effects from natural source. It has been found out that plant having polyphenolic compounds such as flavonoids posses' antioxidant activity (Cook and Samman, 1996) [9]. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity. In the present investigation, phytochemical screening of ethanolic extracts of leaves of *A. marmelos* was revealed for the presence of some phytoconstituents, hence the present study was designed to evaluate antioxidant activity of *A. marmelos.*

2. Materials and Methods

2.1 Chemicals

1, 1-Diphenyl-2-picryl hydrazyl (DPPH), quercetin, catechol, glucose, Anthrone, ascorbic acid, methanol, ethanol and distilled water were obtained from Department of chemistry SHIATS Allahabad,

2.2 Plant material

The leaves of *Aegle marmelos (cv.Narendar-5)* were collected from Allahabad School of Agriculture Department of Horticulture, *SHIATS*. The leaves were dried in the hot air oven at 40°C for 3 days. The dried leaves were ground separately into fine powder using a dry grinder. Out of the total powder obtained 15g dried powder of *Aegle marmelos* plant's leaves were weighed and transferred into conical flask for extract preparation in 100ml solvents (methanol, ethanol and aqueous) and the mixture was shaken and left for 24 hours undisturbed at 4 °C until required for Phytoconstituent determination and Antioxidant activity.

2.3 Phytoconstituent Estimation

Based upon the qualitative analysis the ethanolic extract was considered as a potential extract and taken for further phases of the study. (Umadevi *et al.* (2011) [24].

2.4 Determination of total phenols

The amount of phenolic compounds in the extracts was determined by the Folin Ciocalteu

Colorimetric method (Mc donald *et al*; (2001) and calculated from a calibration curve obtained with catechol as standard. 5ml of Folin Ciocalteu reagent and 4ml of aqueous sodium carbonate were added to 0.5ml of extract (1:10g/ml). After 15 minutes of incubation at room temperature, the absorbance was read at 765nm in a UV- Visible Spectrophotometer. The phenol content was expressed in mg/g.

2.5 Determination of total flavonoids

Aluminium chloride colorimetric method was used for flavanoids determination (Chang *et al;* (2002) [8]. Each extracts (1mg/ml) was prepared in 70 : 30% ethanol and 0.5ml of each sample was separately mixed with 5ml of ethanol, 0.1ml of 10% aluminium chloride,0.1ml of 1M potassium acetate and 2.8ml of distilled water were added and kept at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared using quercetin. The flavonoid content was expressed in mg/g.

2.6 Determination of tannins

Total tannin determination was done by a method elucidated by peri and pompei (1971) [17]. 1 ml of sample extract of concentrations (1mg/ml) was taken in a test tube. The volume was made up to 1 ml with distilled water and 1ml of water serves as the blank. To this 0.5 folin phenol (1:2) reagent was mixed followed by addition of 5 ml of 35% sodium carbonate. This was kept at room temperature for 5 minutes. Blue colour was formed. The colour intensity was ready at 640nm.A standard graph of tannins (tannic acid concentration (1mg/ml) was plotted from where the tannins content of the extract was determined. The total tannin content is expressed in mg/g of the reference used.

2.7 Determination of total carbohydrate content

Total carbohydrate content was estimated by using (Hedges and Hofreiter, 1962) Method. About 100 mg of leaf sample was weighed in a boiling tube. It was hydrolysed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCl and then cooled to room temperature

Solid sodium carbonate was used for neutralization until effervescence ceased the volume was made up to 100 ml and centrifuged at 8000 rpm for 20 minutes the supernant was collected and aliquots of 0.5 and 1 ml were transferred to the test tubes for analysis. Standard was prepared by taking 0 to 0.8 mg/ml as working standard and '0'served as a blank. The volume was made up to 1 ml in all the test tubes including the sample tubes by adding distilled water. Then 4 ml of Anthrone reagent was added to each test tube and heated for eight minutes in boiling water bath. It was then cooled rapidly and read at 630 nm. A standard graph was drawn. The total carbohydrate content was measured in terms of glucose equivalent.

3. Antioxidant activities assay (Spectrophotometric analysis) of *Aegle marmelos*

3.1 DPPH free radical scavenging assay

The DPPH radical scavenging activity assay elucidated by Chang *et al*. (2007) was followed**.** Dilution of the extracts (800 µg/ml) was prepared for different extracts. Then, 1 ml of extract from this dilution was added into the test tube containing 2 ml of DPPH solution Control was prepared by adding 1 ml of methanol to 2 ml of DPPH solution. Ascorbic acid was used as standards. The mixture was shaken vigorously and was left to stand in the dark for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517nm.

3.2 Reducing power assay

Antioxidant activity by reducing power assay elucidated by Yen and Duh (1993) was followed**.** The reducing power of the test sample was determined by taking 800 µg/ml concentrations of the leaf extracts in 1ml methanol. This was mixed with 2.5ml of phosphate buffer and 2.5ml of potassium ferric cyanide in test tubes. The mixture was incubated for 20 min. at 50 °C. At the end of the incubation 2.5ml of tri-chloroacetic acid was added to the mixture followed by centrifugation at 500 rpm for 10 min. The upper layers (2.5ml) were mixed in 2.5ml of distilled water and 0.5ml of ferric chloride and the absorbance was measured at 700nm. The reducing power tests were run in triplicates. Increase in absorbance of the reaction mixture indicated the reducing power of sample.

4. Results and Discussion

4.1 Quantitative estimation of Phytoconstituents

Over the years, the study on medicinal plants to reveal the mechanism of action and to justify their claims by traditional healers has been increase. An angle of this research has been the study of bioactive components and antioxidant properties of the *A. marmelos*. The present study has verified that remedial plants could be good source of antioxidant substances. It has been acknowledged that flavonoids show significant antioxidant action on human health and fitness. The flavonoids act through scavenging or chelating process (Kessler *et al*., 2003; Cook and Samman, 1996) [11, 9]. The high potential of phenolics to scavenge free radicals may be due to many phenolic hydroxyl groups they posses (Sawa *et al*., 1999) [20]. The content of total phenols were measured by Folin -ciocalteu method in terms of catechol standard (the standard curve equation: $y = 0.000688x + 0.6090$, $r^2 = 0.9677$) were found to be 40.836±0.001mg/g (Table 1) of extract. The

contents of total flavonoids that were measured by aluminium chloride colorimetric technique in term of quercetin equivalent (the standard curve equation: y=0.00150x+0.2295, r^2=0.9634) was found to be 66.486±0.005mg/g (Table 1). The total tannin content in terms of tannin acid equivalent (the standard curve equation: y=0.0047x+0.0863, r^2=0.9287 was also observed to be 60.267 ±0.002mg/g (Table 1) of extract. The total carbohydrate in terms of glucose equivalent (the standard curve equation: y=0.00130x+0.3430, r^2 =0.9771 was found to be 34.53±0.570mg/gm (Table 1) of extract.

Table 1: Total phytoconstituent estimation in *Aegle marmelos* leaves extract.

S. NO	Assay	*Aegle marmelos* ethanolic leaf extract in (mg/g)
1.	Phenol	40.836±0.001
2.	Flavonoid	66.480±0.005
3.	Tannin	60.267±0.002
4.	Carbohydrate	37.530±0.570

Each value in the table was obtained by calculating the average of three experiments ± SEM.

5. Free radical scavenging activity

5.1 DPPH assay

Antioxidants are significant in the prevention of human illness and may function as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quencher of singlet oxygen formation (Andlauer and Furst, 1998) [2]. Free radicals posses the ability to reduce the oxidative damage associated with many disease including neurodegenerative diseases, cancer, cardiovascular disease, cataracts and AIDS (Pietta *et al.,* 1998; Lee *et al.,* 2000; Middleton *et al.*, 2000) [18, 13, 16]. Antioxidants through their scavenging power are useful for the management of these diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Blois, 1958) [6] (Figure 1) shows the IC_{50} (µg / ml) values of different plant extracts for free radical scavenging activity by DPPH radical and of the standard Ascorbic acid. The highest radical scavenging activity was shown by Aqueous extract with IC_{50} value =125µg/ml which is higher than Ascorbic acid. The radical scavenging activity of all the three extracts (Aqueous, ethanol and methanol) was determined using DPPH which decreased in the following order: Aqueous (89.12%)>Ethanol (86.10%)>methanol (79.10%) as compared to standard Ascorbic acid (94.61%) for 800µg/ml concentrations.

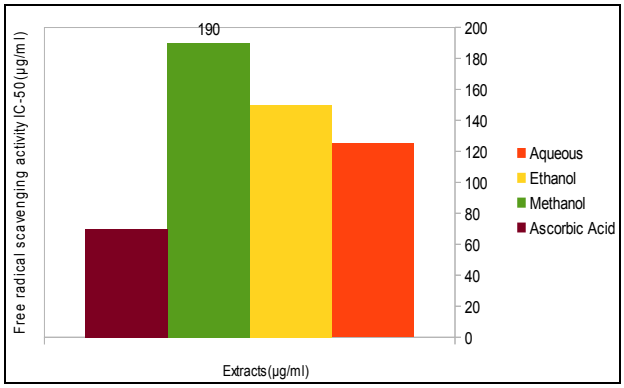

Fig 1: Free radical scavenging activity (IC_{50}) in different extracts of *Aegle marmelos.*

Table 2: Antioxidant activity of different solvent extracts of *Aegle marmelos.*

Extract	RSA (%)(OD517nm)	RPA(OD 700nm)	Concentration (µg/ml)
WE	81.25±0.001	0.062±0.004	800µg/ml
EE	80.14±0.009	0.292±0.020	800µg/ml
ME	79.11±0.006	0.272±0.002	800µg/ml
Ascorbic acid	94.61±0.004	0.792±0.037	800µg/ml

Each value in the table was obtained by calculating the average of three experiments ± SEM.

6. Reducing power assay

In the present study the ability of extracts to reduce Fe (iii) to Fe (ii) was determined and was compared to that of standard ascorbic acid. All the three extracts shoed some degree of electron donating capacity (Table 2) however WE of *Aegle marmelos* showed maximum reduction of Fe (iii) to Fe (ii) indicated higher absorbance at 700nm, but the capacities were lower than that of Ascorbic acid. The order of exhibiting reducing power was similar to that of free radical scavenging. The water extracts of *Aegle marmelos* containing higher polyphenol content exhibited higher electron donating capacity and similar results have been reported in literature (Arabshahi-Delouee and Urooj, 2007, Benzie and Szeto, 1999. Goa *et al.* 2000, Vanitha *et al*; 2012, Savitha *et al*; 2012) [3, 5, 10, 25].

7. Conclusion

On the basis of results obtained it can be concluded that *Aegle marmelos* leaves possess some phytoconstituents and potent antioxidant activities, therefore it inhibits production of free radicals. Further. The potential of this plant be explored more and more, in order to develop an alternative therapy for treatment of various diseases. The present study also suggests the use of this medicinal plants may be exploited for health supplements. Thus justifying its traditional use.

8. References

1. Akindele AJ, Adeyemi. Anti-inflammatory activity of the aqueous leaf extracts of *Byrsocarpus coccineus.* J. Fitoterapia. 2007; 78:25-28.
2. Andlauer W, Furst P. Antioxidative power of phytochemicals with special reference to cereales. Cereal Foods World. 1998; 43:356-359.
3. Arabshahi-Delouee S, Urooj A. Antioxidant properties of various solvent extracts of Mulbery (*Morus indica L)* leaves. Food chemistry. 2007; 102:1233-1244.
4. Barlow SM. Toxicological aspects of antioxidants used as food additives in food antioxidants, Hudson BFJ(ed) Elsevier, London. 1990, 253-307.
5. Benzie IFF, Szeto YT. Total antioxidant capacity of teas by ferric reducing power assay. Journal of Agricaltural Food chemistry. 1999; 47:633-636.
6. Blios MS. Antioxidant determination by the use of a stable free-radical. Nature. 1958; 26:1199-1200.
7. Branen AL. Toxicology and biochemistry of butylated hydroxyanisol and butylated hydroxytoluene. Journal American Oil Chemical Society. 1975; 5:59-63.
8. Chang C, Yang M, Wen H, Chem J. Estimation of total flavonoid in propolis by two complementary colorimetric method. Journal of Food drug analy. 2002.
9. Cook NC, Samman S. Flavonoids-Chemistry, metabolism, cardioprotective effects, and dietary sources. Nutrition. Biochemistry. 1996; 7:66-76.*sis.*10:178-183

10. Goa X, Bjork L, traJkovski V, Uggla M. Evaluation of Antioxidant Activities of rosehip ethanolic extract in different test systems. Journal of Agricultural Food chemistry. 2000; 80:2021-2027.
11. Kessler M, Ubeau G, Jung L. Anti-and pro-oxidant activity of rutin and quercetin derivatives. J. Pharm. Pharmacol. 2003; 55:131-142.
12. Kumpulainen JT, Salonen JT. Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease. The Royal Society of Chemistry. 1999, 178-187.
13. Lee KG, Mitchell AE, Shibamoto T. Determination of antioxidant properties of aroma extracts from various beans. J. Agric. Food Chem. 2000; 48:4817-4820.
14. Mantle D, Eddeb F, Pickering AT. Comparison of relative antioxidant activities of British medicinal plant species *in vitro*, J. Ethnopharmacol. 2000; 72:47-51.
15. McDonald S, Prenzel PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food chemistry. 2000; 73:73-84.
16. Middleton E, Kandaswami C, Theoharides TC. The effects of plants flavonoid on mammalian cell: implication for inflammation, heart disease, and cancer. Pharmacol. Rev. 2000; 52:673-751.
17. Peri C, Pompei C. Estimation of different phenolic groups in vegetable extracts. Phytochemistry. 1971; 10:2187-2189.
18. Pietta P, Simonetti P, Mauri P. Antioxidant activity of selected medicinal plants. J. Agric. Food Chem. 1998; 46:4487-4490.
19. Sauria E, Hassan F. Antioxidant activity of some furanocoumarins isolated from *Heracleum persicum,* J Pharma. Bio. 2007; 42:396-399.
20. Sawa T, Nako M, Akaike T, Ono K, Maeda H. Alkylperoxyl radical scavenging activity of various flavonoids and other phenolics compounds: Implementations for the antitumor promoter effect of vegetables. J. Agric. Food Chem. 1999; 47:397-492.
21. Schuler P. Natural antioxidants exploited commercially, In Food Antioxidants, Hudson BJF (ed.). Elsevier, London. 1990, 99-170.
22. Sen CK. Oxygen toxicity and antioxidant: state of the art. Indian Journal of Physiology and Pharmacology. 1995; 39(3):177-96.
23. Shyura LF, Tsung HJ, Chen J, Chiu CY, Lo CP. Antioxidant properties of extracts from medicinal plants popularly used in Taiwan. Int. j. App. Sci and Eng. 2005; 3(3):195-202.
24. Umadevi KJ, Vanitha Varadharajan, Vijayalakshmi Krishnamurthy. Physicochemical evaluation, phytochemical screening and chromatographic fingerprint profile of *Aegle marmelos* leaf extract. World Journal of Pharmaceutical Research. 2011; 1:813-837.
25. Vanitha Reddy, Sahana, Asna Urooj. Antioxidant activity of *Aegle marmelos* and *Psidium guajava* leaves. International journal of medical aromatic plants. 2012; 2:155-160.