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Full Length Research Paper

Evaluation of antioxidant activity, quantitative estimation of phenols and flavonoids in different parts of *Aegle marmelos*

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In existing study, we carried out an efficient record of the comparative antioxidant activity in methanolic extract of the selected parts (leaves, root and stem bark) of *Aegle marmelos*. Total content of phenol and flavonoid was quantitatively estimated in different parts of *A. marmelos*. The total phenolic contents varied from 9.8367 ± 0.0235 to 1.7281 ± 0.049 mg g^{-1}. Total flavonoid contents were between 8.248 ± 0.029 to 1.087 ± 0.002 mg g^{-1}. Free radical scavenging activity of different extracts was evaluated by using DPPH (1, 1 -Diphenyl- 2 -picryl hydrazyl) method. The highest free radical scavenging effect was observed in leaves with IC_{50} = 2.096µg ml^{-1}. The effectiveness of radical scavenging activity of leaves extract was about 10 times greater than reference antioxidant butylated hydroxy toluene (BHT). The greater amount of phenolic compounds leads to more powerful radical scavenging effect as shown by methanolic extract of *A. marmelos* leaves.

Key words: *Aegle marmelos,* antioxidant, flavonoids, phenols, 1, 1 –diphenyl - 2 -picryl hydrazyl.

INTRODUCTION

In recent years, considerable interest has been evinced by the public and the medical professional regarding the use of indigenous drugs in the treatment of diseases. Several members of the family Rutaceae are being used traditionally for a wide variety of ethnomedical properties. *Aegle marmelose* (L) (Rutaceae) is 1 among them found in India. *A. marmelose* generally acknowledged as bael or koovalam (Malayalam, India) growing wild through out deciduous forest of India, climbing to a height of 1,200 m in Western Himalayas and also occurring in Andaman Island. Its fruits and leaves are valued in indigenous medicine (Charakbraty et al., 1960). The plant has been employed for long time in folk therapy. Poultice made of leaves are used for ophthalmia and ulcers. The leaves are use to lowering the blood glucose levels (Ayurvedic Pharmacopoeia of India, 1988). Other actions like anti-fungal (Renu, 1983), antibacterial (Banerji and Kumar, 1980), antiprotozoal (Banerjee, 1980), antispermatogenic (Sur et al, 1999) are also reported. The plant has been found to contain number of phytoconstituents like aegeline, agelinine, rutin, sterol (Chatterjee and Bose, 1952), β-sitosterol, β -D-glucoside, marmesinine (Sharma et al, 1980), lupeol (Patra et al., 1979), tannins, phlobatannins, flavonoids, umbelliferone, quercetin and volatile oils (Eugenol and methyl eugenol) (Banerjee, and Nigam, 1979). It has been reported that leaves possess cardiotonic effect, antifungal, analgesic and antioxidant activities (Rai, 1996). No scientific evaluation of antioxidant activity of *A. marmelose* has been reported so far. Therefore, it was thought worthwhile to evaluate antioxidant activity of *A. marmelos* to confirm its folk medicine claim. Many naturally occurring products have been reported to contain large amount of antioxidant other then vitamin C, E and carotenoid (Javanmardi et al., 2003). These anti-oxidant play a vital role in delaying, intercepting or preventing oxidative reactions, catalyse by free radical (Vilioglu et al., 1998). This antioxidant activity might be due to the presence of phenolic compounds such as

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flavonoids (Pieatta, 1998), phenolic acids and phenolic diterpine (Shahidi and Wanasundara, 1992). 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). 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). Synthetic antioxidants like butylated hydroxy anisole (BHA, butylated hydroxy 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). Hence, search for natural antioxidant has greatly been increased in the recent scenario (Jayaprakasha et al., 2003). In the literature many crude extracts and pure natural compounds have been reported which have potent antioxidant potential (Schuler, 1990; Chu, 2000; Koleva et al., 2002; Mantle et al., 2000; Oke and Hamburger, 2002). 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). Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski et al., 1987). In the present investigation, phytochemical screening of methanolic extracts of different parts of *A. marmelos* revealed the presence of phenolic and flavonoids compounds. Hence the present study was design to evaluate antioxidant activity of *A. marmelos.*

MATERIALS AND METHODS

Chemicals

1, 1-Diphenyl-2-picryl hydrazyl (DPPH), rutin and ascorbic acid were purchased from Sigma Chemical Co. (St., Louis, USA). Tert-butyl-4-hydroxy toluene (BHT), gallic acid, Folin Ciocalteu reagent, and methanol were purchased from Merck Co. (Germany). All the chemicals and reagents used were of analytical grade.

Plant materials

Different parts of *A. marmelos* were collected from campus of Hamdard University, New Delhi, India, (July 2007), which was identified by Taxonomist, Department of Botany, Hamdard New Delhi. The voucher specimens were deposited in the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard (JHFP, 2023). Plants materials were shade dried at room temperature and ground in a mortar. 25g of each parts of *A. marmelos* powder were extracted in 250 ml of methanol by maceration for 48 h (yield-18.5%). The extracts were concentrated in vacuo at 50°C and the extracts were freeze dried.

Total phenols estimation

The total phenols of all extracts were measured at 765 nm by Folin Ciocalteu reagent (McDonald et al., 2001). The dilute methanolic extract (0.5 ml of 1:10 g ml^{-1}) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous sodium carbonate (4 ml, 1 M). The mixture was allowed to stand for 15 min and the total phenols were determined by spectrophotometer at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg l^{-1} solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of dry mass), which is a common reference compound.

Total flavonoids estimation

Aluminium chloride colorimetric technique was used for flavonoids estimation (Chang et al., 2002). Each extract (0.5 ml of 1:10 g ml^{-1}) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm with a double beam UV/Visible spectrophotometer, SHEMADZU (Japan). The calibration curve was plotted by preparing the quercetin solutions at concentrations 12.5 to 100 g ml^{-1} in methanol.

Free radical scavenging activity

DPPH assay

The free radical scavenging activity of the different parts of *A. marmelos,* butylated hydroxy toluene (BHT), rutin and ascorbic acid was measured in terms of hydrogen donating or free radical scavenging ability by using the stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) (Blois , 1957). Different concentration of me-thanolic plant extract (0.5 ml) was mixed in a test tube with a mixture of 2.5 ml of methanol and 75 µM DPPH, (stable free radical) and record the absorbance at 517 nm. The reaction mixture was set-aside in the dark at room temperature for 90 min and absorbance was recorded at 517 nm. The experiment was done in triplicate. BHT, rutin and ascorbic acid were used as standard controls. IC_{50} value is the concentration of sample, required to scavenge 50% of DPPH free radicals.

Statistical analysis

The statistical significance between antioxidant activity values of the extracts were evaluated by analysis of variance (ANOVA) followed by Dunett's test. P values less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Flavonoid and total phenol contents of the methanolic extracts

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). The high potential of phenolics to scavenge free radicals may be due to many phenolic hydroxyl groups they posses (Sawa et al., 1999). The contents of total flavonoid that were measured by aluminium chloride colorimetric technique in term of quercetin equivalent (the standard curve equation: $y = 0.006x + 0.007$, $r^2 = 0.999$) were between 1.087 ± contents in the extract of leaves (8.248 ± 0.029 mg kg^{-1}) and stem (1.400 ± 0.029 mg kg^{-1}) were higher than that in the extracts of root (1.087 ± 0.002 mg kg^{-1}) (Table 1). Table 2 also show the contents of total phenols that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: $y = 0.004x + 0.003$, $r^2 = 0.991$). The total phenol varied from 1.7281 ± 0.049 to 9.8367 ± 0.02335 mg kg^{-1}. The total phenol in methanolic extract of the leaves (9.8367 ± 0.0235 mg kg^{-1}) and in stem extract (7.4693 ± 0.047 mg kg^{-1}) were higher than that in the extracts of root (1.7281 ± 0.049 mg kg^{-1}). Table 2 shows the comparison of DPPH free radical inhibitory concentration of the plant extracts and those of BHT, ascorbic acid and rutin. The % inhibition of leaf (64.12± 0.01), stem (76.883± 0.03)and root are (64.193± 0.05) in comparison to BHT(65.09± 0.22), ascorbic acid (52.163± 0.02) and rutin (72.686± 0.560) respectively. The compounds such as flavonoids, which hold hydroxyls groups, are responsible for the radical scavenging activity in the plants (Das and Pereira, 1990; Younes, 1981).

Table 1. Total phenolic and flavonoid contents of different parts extract of *A. marmelos*.

Plant parts	Total phenolic contents (mg/kg)	Total flavonoid contents (mg/kg)
Leaf	9.8367 ± 0.0235	8.248 ± 0.029
Stem	7.4693 ± 0.047	1.400 ± 0.029
Root	1.7281 ± 0.049	1.087 ± 0.002

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

Table 2. Comparison of DPPH free radical inhibitory concentration of the plant extracts and those of BHT, ascorbic acid and rutin.

Plant parts	Concentration (µg/ml)	% inhibition
Leaf	3.5	64.12 ± 0.01
Stem	3.5	76.883 ± 0.03
Root	3.5	64.193 ± 0.05
BHT	35	65.09 ± 0.22
Ascorbic acid	35	52.163 ± 0.20
Rutin	35	72.686 ± 0.56

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

Antioxidant activity

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). 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). 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). Figure 1 shows the IC50 (µg ml-1) values of plant extracts for free radical scavenging activity by DPPH radical. IC_{50} of the standard compounds, BHT, ascorbic acid and rutin were 18.726, 29.338 and 12.231µg ml^{-1}respectively. The highest radical scavenging activity was shown by leaf extract with IC_{50} = 2.096µg ml^{-1} which is higher than that of BHT ($P < 0.05$). The radical scavenging activity in the plant extracts decreased in the following order: Leaves > stem > root. The radical scavenging effect of leaves at 3.5 µg ml^{-1} was similar to BHT at 35 µg ml^{-1}. Therefore, the antioxidant effect of leaves extract was 10 times greater than that of the synthetic antioxidant, BHT.

Conclusion

The result of the present study revealed that the leaf extract of *A. marmelos*, which hold maximum amount of flavonoid and phenolic compounds, exhibited the best antioxidant activity. Despite widespread use of *A. marmelos* as folklore medicines in India, the literature contains few reports on its antioxidant activity. In present experiment, we conceded out a systematic record on the relative free radical scavenging activity in methanolic extract of selected parts of *A. marmelos*. We have also established the relationship of total flavonoid and phenolic contents by means of free radical scavenging activity.

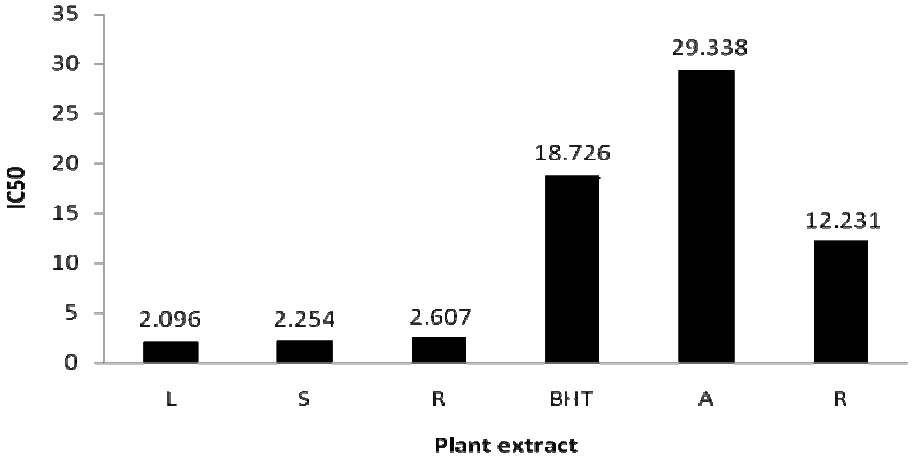

Figure 1. IC_{50} (μgml^{-1}) values of plant extracts for free radical scavenging activity by DPPH radical. Lower IC_{50} value indicates higher antioxidant activity. Extracts: L = Leaves, S = Stem, R = Root, BHT = Butylated hydroxyl toluene, A = Ascorbic acid and R = Rutin.

In this study, *A. marmelos* recognized as having high levels of antioxidant activity. Leaf extract of *A. marmelos* showed higher scavenging property it may be due to the present of hydroxyl groups existing in the phenolic and flavonoid compounds chemical configuration that can provide the essential constituents as a radical scavenger. Free radicals have been reported to be liable for the cataract formation, oxidative damage in the occurrence and development of vascular diseases (Langsethm, 1995; Alho and Leinonin, 1999). Free radi-cal mediated processes have been implicated in the pa-thogenesis of most of the diseases. It is well documented that free radicals take part in the pathogenesis of a large number of diseases (Gyamfi et al., 1999). The present study showed that all extracts demonstrated different extent of antioxidant activity. It was also shown that leaf extract showed significantly higher antioxidant activity than BHT, rutin and ascorbic acid in scavenging of DPPH free radical. This may be support to the high amount of flavonoid and phenolic compounds in the mehanolic extract of *A. marmelos*.

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