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Extraction of Polyphenol, Flavonoid from *Emblica officinalis, Citrus limon, Cucumis sativus* and Evaluation of their Antioxidant Activity

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ABSTRACT

The antioxidant potential of three commonly used edible fruits and vegetables *Emblica officinalis* (Amla)*, Citrus limon* (lemon) and *Cucumis sativus (cucumber)* available in local market of Jaipur (India), was estimated. The parts of food material taken for this study is edible part of *Emblica officinalis,* peel of *Citrus limon* and *Cucumis sativus.* The total phenolic content, antoxidant activity and flavonoid content was estimated by folin-ciocalteau method, phosphomolybdenum assay and aluminium chloride colorimetric technique. All the three activities determined were found maximum in *Emblica officinalis*, but *Citrus limon* and *Cucumis sativus* also show good amount of these activities. Thus the study revealed that the peel of *Citrus limon* and *Cucumis sativus* (otherwise waste) can also be used as a good source of antioxidant activity.

Keywords: Antioxidant, Polyphenol, Flavonoid, Amla, Lemon Peel, Cucumber Peel.

INTRODUCTION

The biological antioxidants are compounds that protects biological systems against the potentially harmful effects of processes or reactions that causes excessive oxidation. Nowadays the oxidative stress is one of the serious problems of the modern society. Different air pollutions, smoking, UV radiation etc. leads to oxidative stress of cells, causes various diseases like dermatitis, melanomas or photo aging of the skin, cancer, heart disease, inflammation, arthritis, immune systems decline, brain dysfunction and cataracts etc. The consumption of fruits and vegetables has been found to be associated with lowering of these diseases as they contain a large amount of phenolic compounds, antioxidants and flavonoids. In various studies it has been found that antioxidants can inhibit or delay the oxidation of an oxidisable substrate in a chain reaction hence antioxidants seem to be very important in prevention of these diseases (Ali *et al.,* 2010; Ames 1983; Ames *et al.,* 1993; Feskanich *et al.,* 2000; Halliwell 1996). The extraction of phenolic compounds can be defined as the separation of medicinally active portion from plant tissues using selective solvents through standard extraction procedures. The main criteria of extraction technique is separating the soluble and insoluble components and leaving behind only insoluble Cellular mass. The extraction

products of plants have relatively complex mixtures covering a number of groups of plant metabolites either in liquid form or semi-solid state (Imran *et al.,* 2011; Ramamoorthy *et al.,* 2007). The general techniques of extraction of medicinal plants include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (soxhlet), aqueous-alcoholic extraction by fermentation, counter current extraction, microwave assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction (SFE), phytonic extraction (with hydro-flouro-carbon solvents), etc. For the aromatic plants, three types of hydro-distillation techniques (water distillation, steam distillation, steam and water distillation), hydrolytic maceration followed by distillation technique, expression method and enfleurage method (cold fat extraction) can be used. Some of the latest methods of extraction for aromatic plants include head space trapping technique, solid phase micro-extraction, protoplast extraction technique, micro-distillation, thermo-micro-distillation, and molecular distillation techniques (Ics-Unido 2006).

The aim of this study is to find out the potential of fruit & vegetables and their waste in terms of their total phenolic, flavonoid content & antioxidant activity. In order to determine these activities a comprehensive study was carried out for 3 plant species collected from local market of Jaipur. The fruits selected were *Emblica officinalis, Citrus limon* and *Cucumis sativus.*

MATERIALS AND METHODS

Materials

Three different types of fruits & vegetables were purchased from local market of Jaipur, Rajasthan (India). These include Amla (*Emblica officinalis*), Lemon (*Citrus limon*) and Cucumber (*Cucumis sativus*). For the present study the parts of fruit taken are, edible part of *amla*, peel of lemon and peel of cucumber. Folin-Chiocalteu reagent, Sodium carbonate, Gallic acid, Quercetin, Methanol, Aluminium chloride, Potassium acetate, Butylated Hydroxy Toluene (BHT), Sulphuric acid, Sodium phosphate, Ammonium molybdate of AR grade were purchased from local supplier of Jaipur.

Sample preparation

All the fruits, vegetables taken were washed with distilled water and desired parts were dried in oven at 70 ^{o}C. After that the parts were grinded and sieved with mesh aperture size of 2 mm. The portion of fruits (1gm) was homogenized with different concentrations of aqueous methanol solution (0-70% methanol). The homogenate was stir with magnetic stirrer at 900 rpm at room temperature for 30 minutes. The extract was centrifuged at 3000 rpm for 20 minutes. The supernatant was removed and filtered with whatmann filter paper 4. Finally the supernatant was stored in the dark. Similarly another extraction was done keeping the methanol**:** water (50**:**50 v/v) ratio constant but varying the amount of sample dose (1gm, 1.5 gm, 2.0gm, 2.5gm).

Determination of total phenolic content

The total phenolic content of the fruit and vegetable extracts were estimated by the method described by Singleton and Rossi (1965) with slight modification and reported as Gallic acid equivalent (GAE) per gram of fruit/vegetable. Gallic acid in various concentrations (20-140 5ØßG/ml) was prepared in methanol: water (50:50 v/v) as standard. 0.5 ml of different extracts was taken in test tubes and 5 ml of Folin-ciocalteau reagent and 4 ml of aqueous sodium carbonate (7.5%) were added to the tubes. The tubes were kept at room temperature for 15 min. The optical density was measured at 745 nm using UV spectrophotometer (UV-1800,Shimadzu).

Determination of antioxidant activity

The antioxidant activity of fruit and vegetable extracts were estimated by phosphomolybdenum assay and reported as butylated hydroxy toluene equivalent (BHTe) per gram of fruit/vegetable. Different concentrations of butylated hydroxy toluene (25-125 µg/ml) in distilled water were prepared as standard. 0.3 ml of fruit extracts were taken in test tubes. The reagent solution was prepared by mixing 10 ml of 0.6 M sulphuric acid, 10 ml of 28 mM sodium phosphate and 10 ml of 4 mM ammonium molybdate into a beaker and 3 ml reagent solution was added to all the tubes. 0.3 ml of methanol served as blank. All the tubes were incubated at 95 ^{o}C for 90 min. The

tubes were cooled to room temperature and the optical density was measured at 695 nm using UV spectrophotometer (UV-1800,Shimadzu).

Determination of total flavonoid content

The total flavonoid content of the fruit and vegetable extracts were estimated by using aluminium chloride colorimetric technique and reported as Quercetin equivalents (Qe) per gram of fruit/vegetable. Quercetin in various concentrations (12.5-100 ìg/ml) was prepared in methanol as standard. 0.5 ml of each methanol extracts (1:10) were taken in test tubes and 1.5 ml methanol, 0.1ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml distilled water were added separately to each tubes. All the tubes were incubated at room temperature for 30 min. optical density was measured at 415 nm by using UV spectrophotometer (UV-1800,Shimadzu).

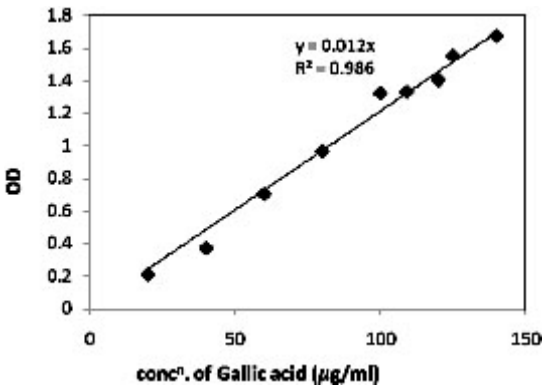

Fig 1. Standard curve for determination of Total phenolic content

RESULTS AND DISCUSSION

The total polyphenol content, antioxidant activity and flavonoid content was determined by folin-ciocalteau method, phosphomolybdenum assay and aluminium chloride colorimetric technique respectively as stated above.

Total Polyphenol content

The standard curve (y = 0.012 x, r^2 = 0.986) for determination of total polyphenol content was obtained by measuring OD of standard solution of Gallic acid and is shown in Fig 1. The Total phenolic content of all the three samples/extracts were then estimated using this standard curve and results were shown in Fig 2.

Effect of solvent Concentration

It can be seen from the Figure 2.a that polyphenol content of *Emblica officinalis,* extract increases with increase in concentration of methanol in the solvent. The maximum amount of polyphenol extracted was 112.52 mg GAE/g at 70% methanol in water nearly equal to amount (111.72 mg GAE/g) obtained at 50% methanol in water. Similarly *Citrus limon* (peel) and *Cucumis sativus* (peel) also shows an increase in total polyphenol content with increase in concentratuion of methanol but the increase is not very high after 50% methanol concentration. The maximum amount of polyphenol extracted was 9.7 and 7.4 mg GAE/g for lemon peel and cucumber peel respectively at 70% methanol

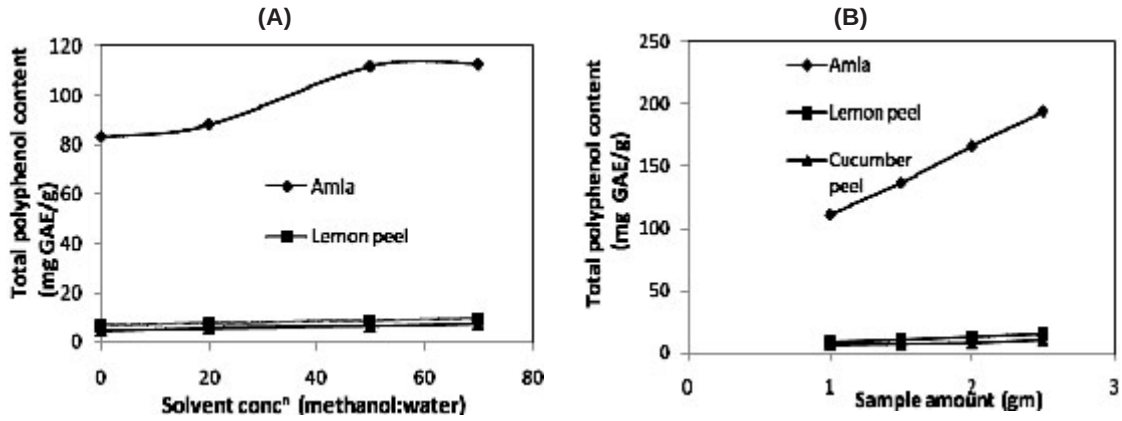

Fig 2 (A) Effect of solvent concentration (B) Effect of amount of sample on extraction of total polyphenolics compounds Total Flavonoid content

in water. Therefore, 50% methanol in water was taken as optimum solvent concentration for further study.

Effect of amount of dry sample powder

The 50% methanol solution was used to extract total polyphenol from all the three samples by varying the amount (1-2.5 g) of dry powder of fruit/vegetable, the results obtained were shown in Figure 2.b. It can be seen from the figure that total phenolic content of extract increases with increase in amount of dry sample. For 2.5 g of sample it is 194, 16.072, 10.0548 mg GAE for amla, lemom and cucumber peel respectively. It can be seen that *Emblica officinalis* shows maximum phenolic content as actual fruit part is taken for study. Both *Citrus limon* and *Cucumis sativus* shows comparable amount of phenolic content as waste part was taken for the study (peel). Though amla contains very good amount of phenolic compounds, the amount of phenolic compounds in lemon and cucumber peels are good enough to extract. Therefore these materials thrown away everyday from households as waste can be used for extraction of valuable phenolic compounds.

The standard curve ($y = 0.005\ x$, $r^2 = 0.994$) for determination of total flavonoid content was obtained by Aluminium chloride colorimetric technique as Quercetin equivalents and is shown in Fig 3. The Total flavonoid content of all the three samples/extracts were then estimated using this standard curve and results were shown in Fig 4.

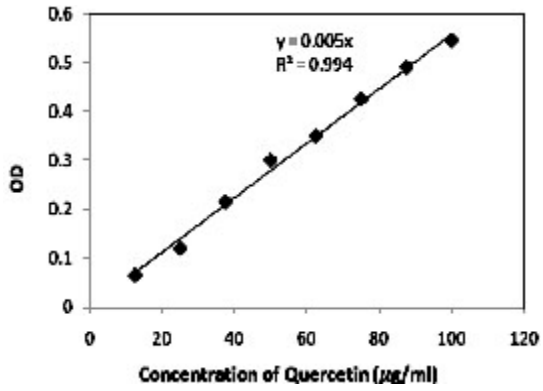

Fig 3. Standard curve for determination of Total flavonoid content

Effect of solvent Concentration

It can be seen from the Figure 4.a that total flavonoid content of all the three sample extract increases with increase in concentration of methanol in the solvent but the increase is not very sharp. The maximum amount of flavonoid extracted was 19.2,18.1,17.1 mg Qe/g of amla, lemon peel, & cucumber peel respectively, at 70% methanol in water nearly equal to amount (111.72 mg GAE/g) obtained at 50% methanol in water. Therefore, 50% methanol in water was taken as optimum solvent concentration for further study. Diankov *et al.,* (2011) also found that solvent concentration does not affect significantly the extraction rate. It can also be seen from the figure that similar trend was obtained all the three samples with respect to solvent concentration and the amount of flavonoid content is also comparable.

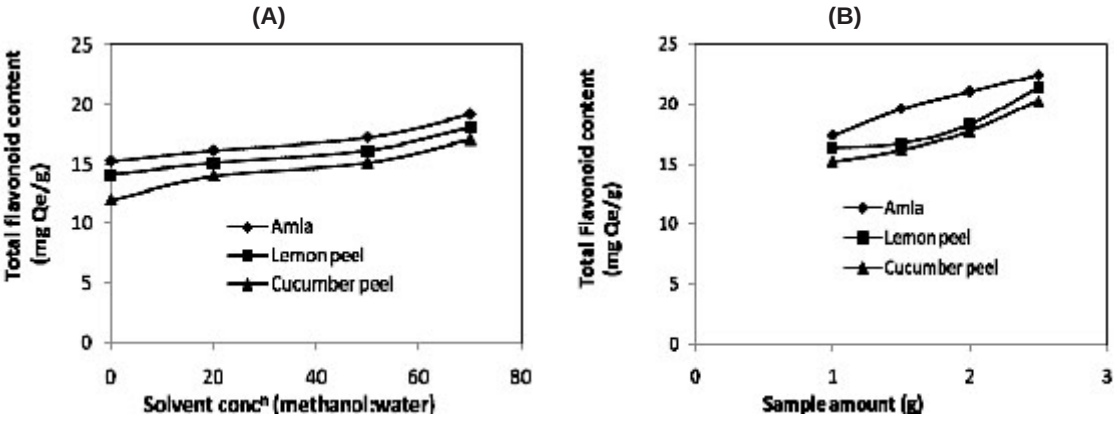

Fig 4. (A) Effect of solvent concentration (B) Effect of amount of sample on extraction of total flavonoids

Effect of amount of dry sample powder

The 50% methanol solution was used to extract total flavonoid from all the three samples by varying the amount (1-2.5 g) of dry powder of fruit/ vegetable, the results obtained were shown in Figure 4.b. It can be seen from the figure that total flavonoid content of extract increases with increase in amount of dry sample. For 2.5 g of sample it is 22.41, 21.34, 20.28 mg Qe for amla, lemom and cucumber peel respectively. It can be seen that in terms of total flavonoid content all the three samples are comparable. Though amla contains higher amount of flavonoids, the amount present in lemon and cucumber peels is nearly equal to it. Therefore, these waste materials can be used for extraction of valuable flavonoids instead of costly amla fruit.

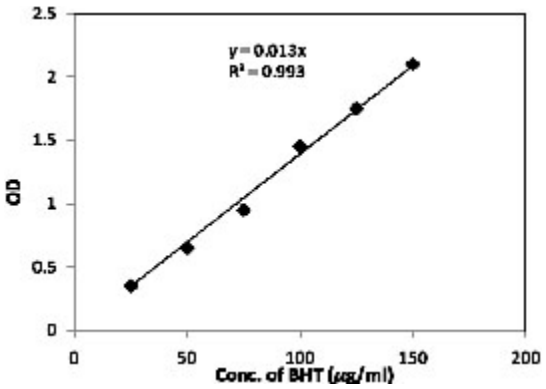

Fig 5. Standard curve for determination of Antioxidant activity

Antioxidant activity

For determination of total antioxidant activity Phospho molybdenum assay and metal chelating activity are reported as butylated hydroxyl toluene (BHT) equivalents. The standard curve (y = 0.013 x, r^2 = 0.992) obtained is shown in Fig 5. The antioxidant activity of all the three samples/extracts was estimated using standard curve and results were shown in Fig 6.

Effect of solvent Concentration

It can be seen from the Figure 6.a that antioxidant activity of all the three sample extract increases with increase in concentration of methanol in the solvent but the increase is not very sharp. The maximum antioxidant activity shown was 8.54, 2.91& 1.75 mg BHTe/g of amla, lemon peel, & cucumber peels respectively, at 70% methanol in water. It can also be seen from the figure that maximum antioxidant activity was shown by amla. Lemon and cucumber pees also show the antioxidant activity therefore these can be used as cheap source of antioxidants.

Effect of amount of dry sample powder

All the three samples were analyzed for their antioxidant activity by varying the amount (1-2.5 g) of dry powder of fruit/vegetable; the results obtained were shown in Figure 6.b. It can be seen from the figure that total antioxidant activity of extract increases with increase in amount of dry sample. For 2.5 g of sample it is 13.132, 3.332, 2.352 mg of BHT/ 2.5 g for amla, lemom and cucumber peel

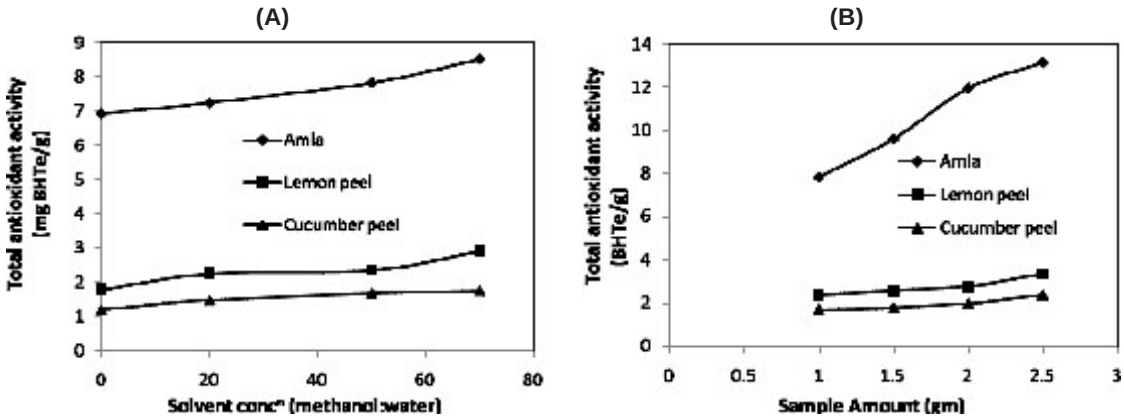

Fig 6. (A) Effect of solvent concentration (B) Effect of amount of sample on antioxidant activity

respectively. It can be seen that in terms of antioxidant activity amla is superior to lemon and cucumber peel. Though amla shows higher antioxidant activity, good activity was also shown by lemon and cucumber peels.

CONCLUSION

In the present study, 50% aqueous solvent extracts from three fruit and vegetable sample gave higher amounts of total polyphenol and antioxidant activity. It can be concluded that polyphenol content is proportional to antioxidant property of samle as amla having higher polyphenol content shows higher antioxidant property compared to lemon and cucumber peels. It can also be concluded that there is no direct relationship between total phenolic compounds present in the sample and the total flavonoid content. Amla having very high amount of total phenolic content shows similar flavonoid content with lemon and cucumber peels. Thus lemon and cucumber peels can be considered as cheap source of flavonoid, and can be used efficiently for its extraction. These materials, otherwise waste can be used as potential source of antioxidants for industrial application also.

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