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# Phytochemical screening and antioxidant potential of some plant materials

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# **ABSTRACT**

Secondary metabolites are the biomolecular wealth of the plants offering them different attributes. Antioxidant activity is one of the most important quality of the plants, which humans are using since time immemorial. Every organism produces number of reactive species of oxygen, nitrogen and chlorine during physiological and environmental influences. Prolonged exposure in even in low concentration causes damage of biologically important molecules causing diseased conditions. The removal such toxic free radicals used to be achieved through enzymatic and non-enzymatic reactions. Numerous studies have shown that phytochemical antioxidants are also capable of removing free radicals. Present investigation is an attempt to explore phytochemical constitution of 13 different plant samples including two wild fruits Aegle marmelos and Kigelia pinnata in five different solvent systems and determination of its antioxidant potential using regular DPPH and H<sub>2</sub>O<sub>2</sub> method. Phytochemical profiling revealed presence of phenolic and polyphenolic compounds exhibiting potent antioxidant activities with solvent wise variations to some extent. A Radical Scavenging Activity of (RSA) the methanol extract for every plant sample uncovered maximum RSA in apple, pear, potato. Wild fruits have also exhibited significant RSA substantiating its application as an antioxidant.

 $\label{eq:condary} \textbf{Keywords}: Antioxidant\ Activity,\ DPPH,\ H_2O_2,\ Fruits\ ,\ Secondary\ Metabolite,\ Radicle\ Scavenging\ Activity,\ Wild$ 

# INTRODUCTION

Fruits contain large number of biomolecules and number of secondary metabolites such as alkaloids, saponin, terpenoids, etc. These secondary metabolites offer medicinal attributes to majority of the plants (Verpoorte, 1998). Generally, most of the secondary metabolites like vitamin C, vitamin B1, vitamin B2, ascorbic acid, phenolic compounds, carotenoids, etc. are the standard antioxidants responsible for antioxidant properties (Zou *et al.* 2016). The structure and composition of flavonoids confers them the best

quality of antioxidant property (Van Acker et al. 1996). Rapid urbanization, increasing population and pollution is reported to be the main causes of threatening health. Medically, most of the diseases posing hazard are arising due to interplay of reactive oxygen species (ROS) in various physiological processes (Halliwell, 1991). Antioxidants are the natural biomolecules, which tackle and destroy these free radicals and scavenge disease (Pham-Huy et al., 2008). Green plants are the chief resource of the antioxidants and hence may be solution to tackle these free radical (Shao et al. 2008), but animals are deprived of such antioxidant and need to be ingested (Blokhina et al., 2003). Currently, huge surging trend is noticed in scientific deliberations exploring antioxidant potential of the plants and thereby reducing the risk of free radicles involved in diseases, ailments and natural degradative processes like aging. Besides well-known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices, some natural antioxidant (e.g. rosemary and sage) are already exploited commercially either as antioxidant additives or nutritional supplements (Pourmorad et al., 2006). There is continuous addition of the plants to the list of plants possessing antioxidant potential. Principally, cultivated plants are considered in several research targets. Wild plants are the ignored group to survey for their antioxidant potentials (Schaich et al., 2015). In view of great source of this natural medicine available with us, there is still exigency to explore plant world for

phytochemical composition and antioxidant prospective. Routinely followed, authentic, easy, rapid and sensitive methods for the antioxidant screening of plant extracts are free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) and hydrogen peroxide (Antolovich et al. 2002; Koleva et al. 2002). In present investigation, systematic study of eleven domestic plant samples from apple, lemon, orange, papaya, Alove vera, turmeric, neem, cucumber, tomato, potato, pear (fruits or leaves or tubers) with two wild plant samples i.e. Aegle marmeloes and Kigelia pinnata was carried out. Phytochemical screening for tannin, saponin, terpenoids, cardiac glycosides, steroids, flavonoides, triterpenoids, glycosides, reducing sugar, phlobatanin, anthraquinones, leucoanthanocynin, fatty acid, caumarins, emodines was done. Antioxidant activity of all the materials was tested by DPPH and H<sub>2</sub>O<sub>2</sub> assay.

# **MATERIALS AND METHODS**

#### **Collection of Plant Samples:**

Cultivated plant materials (Table 1) were collected, identified and validated from department of Horticulture, Shri Shivaji Agriculture College, Amravati, MS, India and wild fruits wild fruit of *Aegle* and *Kigelia* validated in department of Botany, Shri Shivaji Science College, Amravati, MS, India.

Table 1 Plant materials used for study

S.N.	Common name	Scientific name	Family	Organ studied
1.	Apple	Malus pumila	Rosaceae	Fruit
2.	Lemon	Citrus limon	Citraceae	Fruit
3.	Mandarin orange	Citrus reticulate	Citraceae	Fruit
4.	Papaya	Carica papaya	Caricaceae	Fruit
5.	Aloe	Alove vera	Asphodelaceae	Stem
6.	turmeric	Curcuma longa	Zingiberaceae	Rhizome
7.	Neem	Azadirachta indica	Meliaceae	Leaves
8.	Cucumber	Cucumis sativus	Cucurbitaceae	Fruit
9.	Tomato	Lycopersicon esculentum	Solanaceae	Fruit
10.	Potato	Solanum tuberosum	Solanaceae	Fruit
11.	Pear	Pyrus communis	Rosaceae	Fruit
12.	Bel	Aegle marmeloes	Rutaceae	Fruit
13.	Hattifal	Kigelia pinnata	Bignoniaceae	Fruit

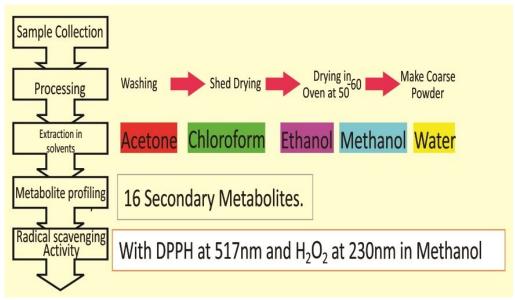


Figure 1 Schematic presentation of methodology

### **Extraction**

- 1) Samples were shed dried for two days followed by Hot Air Oven drying at 50°C to 60°C for next three to four days. After getting stable dry weight grinded to coarse powder.
- 2) Each powdered material was subjected to successive solvent extraction.
- Five different solvents systems viz. water, methanol, ethanol, chloroform and acetone were used to study phytochemical profile
- 4) Proportion of material to solvent was taken as 1:10 (mg/ml). It has been soaked in the above particular solvent and then filtered by Whatmann paper no.1.

# Phytochemical screening

There are number of secondary metabolites present but some important (16 types) of secondary metabolite were studied. The standard methodologies were adopted (Sofowora, 1993; Trease and Evans, 1989; Ahmad, 2007; Evans, 2002; Kadabadi *et al.* 2011).

**Tannins:** 2 ml of extract, added 2 ml FeCl<sub>3</sub>. Colour was changed into blue to black so it shows that presence of Tannin.

**Alkaloids:** 2 ml extract, was hydrolyzed by 1% HCl, then added Mayer's, Wagner's, Dragendorff's reagent individually to each of sample then it produces creamish, brown, red / orange precipitates so this precipitate shows the presence of alkaloids.

**Saponins:** 0.5 ml of extract was added with 5 ml of distilled water, then it was shaked upto 30 sec the

persistent frothing or foam has been form the amount of foam has been indicating presence of saponins.

**Terpenoids (Salkowski test):** 2 ml of Chloroform was added in 0.2 ml of extract and then added concentrated  $H_2SO_4$  from sides of the test tube. Then it appears reddish brown colour at the interface so it shows that presence of terpenoids in extract.

Cardiac glycosides (Keller - Killiani test): Take a 1-2 ml of extract and then added half amount of glacial acetic acid and then added few drops of FeCl3 and concentrated H<sub>2</sub>SO<sub>4</sub> added from the sides of test tube, green, blue precipitate was occurred so it shows the presence of cardiac glycosides.

**Steroids (Salkowski test):** In 0.5 ml extract 2 ml chloroform and concentrated H2SO4 was added from sides of the test tube then it forms lower layer, reddish brown coloration at interface so it shows the presence of steroids.

**Flavonoids:** 0.2 ml of extract dilute sodium hydroxide was added then it creates intense yellow colour, which on addition of HCl it turns into colourless it shows presence of flavonoids.

**Triterpenoids:** Take 100  $\mu$ l extract and mixed with 1 ml of chloroform and later 1 ml of acetic anhydride and then 2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added from sides of the test tube it creating reddish violet color infers, it shows the presence of triterpenoids.

**Glycosides:** Extract was hydrolysed by HCl and neutralized with NaOH then added Fehling's solution A and B in 1:1 proportion, it produces red precipitate, which shows the presence of glycosides.

**Reducing Sugars (Fehling's Test):** Extract was shaken with distilled water and filtered, it was boiled on the addition of Fehling's solution A and B in equal quantity, it appears orange red precipitate which shows the presence of reducing sugars.

**Phlobatanins:** Extract was added with distilled water then shaked and filtered then added 2% HCL and boiled, Red coloured was developed which detect the presence of Phobatannins.

**Anthraquinones (Borntrager's test):** 1 ml extract, added 10 ml benzene then shaken vigorously followed by filtration and then added 5 ml of 10% ammonia and shaked again, the appearance of pink/red/violet coloration in ammonia layer shows the presence of anthraquinones in the extract.

**Leucoanthocyanins:** Extract was added with same amount of isoamyl alcohol, if an upper layer into turns red, it shows that presence of leucoanthocyanins.

**Fatty acids:** Extract was added to same amount of ether then it poured on whatann filter paper in petri dish, after the evaporation of ether filter paper seen transparent, it indicates the presence of fatty acids in extract.

**Coumarins:** 2 ml of extract, added 3 ml of 10% NaOH; Yellow colour was appear which indicates presence of coumarins.

**Emodins:** 2 ml of ammonium hydroxide and 3 ml of benzene then added to the extract, red colour was obtained it indicates presence of Emodins.

# Anti-oxidant activity (AOA)

AOA was investigated for the methanol plant extract only.

**DPPH scavenging activity:** The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts. Different concentrations of each extract were

added, an equal volume, of DPPH (100  $\mu$ M). After 15 min at room temperature, the absorbance was recorded at 517 nm. against Blank containing 5 ml of methanol, and control is containing 1 ml methanol and 4 ml of DPPH. The experiment was repeated for three times.

The % of inhibition is calculated by using formula -

% of Inhibition= 
$$\frac{AC-AS}{AC}$$
 X100

Where, AC is absorbance of control and AS, is absorbance of sample.

**Hydrogen peroxide scavenging activity:** A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extract (20–60 lg/mL) in distilled water was added to hydrogen peroxide and absorbance at 230 nm determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows:

% Scavenged (
$$H_2O_2$$
) =  $\frac{Abr-Aar}{Aar}$  X100

where Abr is the absorbance before reaction and Aar is the absorbance after reaction.

# **RESULTS AND DISCUSSION**

#### Phytochemical screening of plant material

The phytochemical screening results for the plant materials under study are tabulated (Table 2 - 6). The prominent thing in the results is presence of Flavonoids in almost all the samples and all the solvents. The fruits contain maximum types of Secondary metabolites than leaves and tubers except Turmeric, Neem leaves. Other metabolites like tannin, alkaloids, saponins, steroids and caumarins were also detected. Gylcosides were reported only in chloroform and water. Triterpenoids were of rare occurrence, not extracted in chloroform. Leucoanthanocynin, fatty acids were tested but found absent in all the materials. Wild fruits of Aegle and Kigellia also shown presence of many metabolites. Phytochemical investigations of the plants are the routinely conducted studies exploring biochemical wealth of the plants (Lako et al. 2007; Heber 2004). Table 2: Phytochemical screening of plant materials in Acetone solvent system

S.N.	Samples	TN	Alk	Sap	TP	CG	Str	FLV	TTP	GLY	RS	PH	ANQ	LCA	FA	CAU	EMO
1.	Apple	-	-	-	+	-	+	+++	++	-	++	-	-	-	-	-	-
2.	Lemon	-	-	-	++	-	++	+++	++	-	+	-	-	-	-	+++	-
3.	Orange	-	-	-	-	-	-	+++	++	-	++	-	-	-	-	+	-
4.	Papaya	-	++	++	+++	-	+++	+	-	-	+++	-	-	-	-	-	-
5.	Aloe Vera	+	-	-	-	+	-	+	-	-	-	-	-	-	-	++	-
6.	Turmeric	++	-	-	+++	-	+++	++	-	-	-	+++	+++	-	-	-	+++
7.	Neem Leaves	++	-	-	+++	+	+++	++	-	-	++	-	-	-	-	-	-
8.	Cucumber	-	-	+	+	-	+	+	-	-	-	-	-	-	-	++	-
9.	Tomato	-	-	-	+	-	+	+	-	-	+++	++	-	-	-	-	-
10.	Potato	-	-	+	-	-	-	+++	+	-	-	-	-	-	-	-	-
11.	Pear	-	-	-	+	-	+	+	++	-	++	-	-	-	-	++	-
12.	Aegle	-	-	-	+	-	+	++	-	-	-	-	-	-	-	-	-
13.	Kigelia	-	-	-	+	-	+	+	++	-	+	-	-	-	-	+	-

Tannin, Alkaloid, Saponin, Terpenoids, Cardiac Glycosides, Steroids, Flavonoides, Triterpenoids, Glycosides, Reducing Sugar, Phlobatanin, Anthraquinones, Leucoanthanocynin, Fatty acid, Caumarins, Emodines

Table 3: Phytochemical screening of plant materials in Chloroform solvent system

S.N.	Samples	T	Alk	Sap	TP	CG	Str	FLV	TTP	GLY	RS	PH	ANQ	LCA	FA	CAU	EMO
1.	Apple	-	+	-	-	+	-	++	-	-	+	-	-	-	-	-	-
2.	Lemon	-	+	++	-	-	-	+	-	++	+++	-	-	-	-	-	-
3.	Orange	-	+	-	-	+	-	+++	-	-	++	-	-	-	-	+	-
4.	Papaya	-	+	+	-	+	-	+	-	-	++	1	-	-	-	++	-
5.	Aloe vera	-	-	-	-	++	-	+	-	+++	+	1	-	-	-	-	-
6.	Turmeric	-	NA	-	-	-	-	++	-	+++	+	+++	++	-	-	-	++
7.	Neem leaves	-	NA	++	+	++	+	+	-	+++	++	-	-	-	-	-	-
8.	Cucumber	-	-	++	+	-	+	+	-	++	++	1	-	-	-	+	-
9.	Tomato	-	-	-	-	-	-	+	-	-	+	++	-	-	-	-	-
10.	Potato	-	+	-	-	-	-	+	-	-	++	1	-	-	-	-	-
11.	Pear	-	+	+	-	-	-	+	-	-	++	1	-	-	-	-	-
12.	Aegle	-	+	-	-	-	-	+	-	-	++	-	-	-	-	-	-
13.	Kigelia	-	+++	-	-	-	-	+	-	-	++	-	-	-	-	-	-

Tannin, Alkaloid, Saponin, Terpenoids, Cardiac Glycosides, Steroids, Flavonoides, Triterpenoids, Glycosides, Reducing Sugar, Phlobatanin, Anthraquinones, Leucoanthanocynin, Fatty acid, Caumarins, Emodines

Table 4: Phytochemical screening of plant materials in Ethanol solvent system

S.N.	SAMPLES	T	Alk	Sap	TP	CG	Str	FLV	TTP	GLY	RS	PH	ANQ	LCA	FA	CAU	ЕМО
1.	Apple	-	-	-	++	-	++	++	++	-	+	-	-	-	-	++	-
2.	Lemon	-	+++	+	+	-	+	+++	-	-	++	-	-	-	-	++	-
3.	Orange	-	++	-	-	-	-	+++	-	-	++	-	-	-	-	+++	-
4.	Papaya	-	+	+	+++	-	+++	++	-	-	-	-	-	-	-	-	-
5.	Aloe vera	-	++	-	-	-	-	++	-	-	+	-	-	-	-	++	-
6.	Turmeric	++	++	-	+	-	+	++	-	-	-	++	+++	-	-	-	+++
7.	Neem leaves	-	-	+	-	+	-	+++	-	-	++	-	-	-	-	+	-
8.	Cucumber	-	++	-	-	-	-	++	-	-	-	-	-	-	-	-	-
9.	Tomato	-	-	-	+	-	+	++	-	-	+++	-	-	-	-	-	-
10.	Potato	-	-	-	-	+	-	+++	-	-	-	-	-	-	-	-	-
11.	Pear	-	-	-	+	+	+	++	++	-	+	-	-	-	-	+	-
12.	Aegle	-	+	-	+	+	+	++	-	-	-	-	-	-	-	-	-
13.	Kigelia	-	-	-	-	-	-	++	+	-	++	-	-	-	-	-	-

Tannin, Alkaloid, Saponin, Terpenoids, Cardiac Glycosides, Steroids, Flavonoides, Triterpenoids, Glycosides, Reducing Sugar, Phlobatanin, Anthraquinones, Leucoanthanocynin, Fatty acid, Caumarins, Emodines

Table 5: Phytochemical screening of plant materials in Methanol solvent system

S.N.	SAMPLES	T	Alk	Sap	TP	CG	Str	FLV	TTP	GLY	RS	PH	ANQ	LCA	FA	CAU	EMO
1.	Apple	+	+	-	-	-	-	++	++	-	+	-	-	-	-	++	-
2.	Lemon	+	-	+	-	-	-	+++	-	-	++	-	-	-	-	+++	-
3.	Orange	-	++	++	+	-	+	+++	+	-	++	-	-	-	-	++	-
4.	Papaya	-	-	-	++	-	++	++	-	-	+	-	-	-	-	-	-
5.	Aloe vera	-	++	+	-	-	-	++	-	-	+	-	-	-	-	+	-
6.	Turmeric	++	++	-	+	-	+	++	-	-	-	++	+++	-	-	-	+++
7.	Neem leaves	++	++	+	++	-	++	++	-	-	++	-	-	-	-	+	-
8.	Cucumber	-	++	++	-	-	-	+++	+	-	-	-	-	-	-	-	-
9.	Tomato	-	-	-	++	-	++	++	-	-	+++	-	-	-	-	-	-
10.	Potato	-	-	+	-	-	-	++	-	-	-	-	-	-	-	-	-
11.	Pear	-	-	+	-	-	-	+	+	-	++	-	-	-	-	+	-
12.	Aegle	+	-	+++	+++	-	+++	++	-	-	++	-	-	-	-	-	-
13.	Kigelia	+	-	+	++	-	++	++	-	-	++	-	-	-	-	++	-

Tannin, Alkaloid, Saponin, Terpenoids, Cardiac Glycosides, Steroids, Flavonoides, Triterpenoids, Glycosides, Reducing Sugar, Phlobatanin, Anthraquinones, Leucoanthanocynin, Fatty acid, Caumarins, Emodines

Table 6: Phytochemical screening of plant materials in Water solvent system

S.N.	SAMPLES	T	Alk	Sap	TP	CG	Str	FLV	TTP	GLY	RS	PH	ANQ	LCA	FA	CAU	ЕМО
1.	Apple	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
2.	Lemon	-	+	+++	-	-	-	-	+	-	+++	-	-	-	-	+++	-
3.	Orange	++	++	-	-	-	-	-	++	-	++	-	-	-	-	++	-
4.	Papaya	-	++	+	+++	-	+++	-	-	-	+	-	-	-	-	-	-
5.	Aloe vera	-	+	-	-	+	-	-	+	-	-	-	-	-	-	++	-
6.	Turmeric	-	++	-	++	-	++	-	+	-	-	-	+++	-	-	-	+++
7.	Neem leaves	-	++	-	-	+	-	-	+++	-	++	-	-	-	-	+++	-
8.	Cucumber	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9.	Tomato	-	++	-	++	-	++	-	-	-	+++	-	-	-	-	-	-
10.	Potato	-	+	+	-	+	-	-	+	-	++	-	-	-	-	-	-
11.	Pear	-	+	-	+	+	+	-	+	-	++	-	-	-	-	-	-
12.	Aegle	++	+	+++	++	-	++	-	-	++	++	-	-	-	-	-	-
13.	Kigelia	-	-	-	++	-	++	-	-	-	++	-	-	-	-	-	-

Tannin, Alkaloid, Saponin, Terpenoids, Cardiac Glycosides, Steroids, Flavonoides, Triterpenoids, Glycosides, Reducing Sugar, Phlobatanin, Anthraquinones, Leucoanthanocynin, Fatty acid, Caumarins, Emodines

Table 7: Radical Scavenging Activity in Methanol Solvent with DPPH and H<sub>2</sub>O<sub>2</sub> Method

S.N.	SAMPLE	DPPH RSA%	H <sub>2</sub> O <sub>2</sub> RSA%
1.	APPLE	82.296	44.219
2.	LEMON	53.249	25.009
3.	ORANGE	60.742	33.875
4.	PAPAYA	44.695	26.279
5.	ALOVE	38.555	22.682
6.	TURMURIC	60.543	32.495
7.	NEEM	63.305	27.745
8.	CUCUMBER	67.927	38.111
9.	TOMATO	25.450	22.222
10.	POTATO	72.222	42.415
11.	PEAR	88.300	49.868
12.	AEGLE	76.327	43.414
13.	KIGELIA	74.646	41.699

Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (Potterat, 1997; Khedkar and Oke, 2013).

#### **Antioxidant activity**

DPPH and H<sub>2</sub>O<sub>2</sub> tests for AOA (Table 7) shown similar pattern of radicle scavenging activity (RSA). DPPH exhibited slightly greater activity than H<sub>2</sub>O<sub>2</sub>. The fruits which contain maximum RSA may be preferred in diet to get rid of the reactive oxygen species degrading physiological processes. They may be used in future to prepare antiaging drugs. As these plant materials are with diverse composition of secondary metabolites and showing RSA, they are good source of antioxidants and their use in facial treatments in beauty parlors is also justified (Shivanand et al., 2010; Akhter et al. 2008). Apple and pear exhibited maximum RSA. This quality of these fruit recommends daily consumption of dried fruits in order to gain full benefit of essential nutrients, health-promoting phytochemicals, and antioxidants that they contain, together with their desirable taste and aroma. Recently, much interest in the health benefits of dried fruits has led to many in vitro and in vivo (animal and human intervention) studies as well as the identification and quantification of various groups of phytochemicals (Chang et al., 2016). Plant materials from the members of family solanaceae also shown the significant RSA in different solvent systems. Several studies have reported variations in the biological activities of extracts prepared using different extraction techniques (Almoulah et al. 2017; Dhanani et al. 2017; Jimoh et al., 2010). Neem and turmeric are also found prospective in antioxidant properties. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex. More than 140 compounds have been isolated from different parts of neem. All parts of the neem tree are in use to cure different diseases. Neem leaf and its constituents have been demonstrated to exhibit diverse medicinal properties including antioxidant nature (Subapriya and Nagini, 2005). Potential uses of turmeric products as antioxidant and antifungal is also reported (Muthomi et al., 2017). AOA in Aegel and Kigellia reported in this study is the virgin area of investigation undertaken. Earlier research tested both their toxicity and recommended that they are not toxic in nature, the reports of the antifungal, antibacterial, antiinflamatory, anticancer, antianalgic, antidiarrhoeal, etc. activities shown their utility (Rajasekaran, 2014; Ibrar et al., 2007; Megraj et al. 2011).

# **CONCLUSION**

It is evident from the present study that, almost all the plant materials under consideration are great source of secondary metabolites. Variation in the solvent system revealed that, ethanol and methanol are a good solvents, which showed that maximum samples are extracted in them. Therefore, methanol was used to report radical scavenging activity. The flavonoids controlling antioxidant activity is richly found in apple, pear, potato and Aegle. Result of radical scavenging activity showed that apple, pear, potato, Aegle and Kiegelia has maximum radical scavenging property. Therefore, Aegle and Kiegelia are the wild alternatives available in bulk quantity for use in place of apple and pear. The future scope of this research is in production of antioxidants in bulk quantity with the industrial support which will serve the purpose of social relevance also and validation of the method modified in this attempt.

Conflicts of interest: Not declared

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