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# EVALUATION OF *IN VITRO* ANTIOXIDANT CAPACITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF EIGHT DIFFERENT PLANTS MATERIALS

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## **Keywords:**

Phytonutrients, *Phyllanthus emblica*, *Tectona grandis*, *Solanum nigrum*, *Curcuma longa*, Antioxidant.

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**ABSTRACT:** The plants and their materials have been utilized for treatment of various diseases in human and animals which are rich source of antioxidants due to presence of phytonutrients such as vitamins (alpha- tocopherol, Ascorbate, carotenoid) or minerals (zinc, selenium) and flavanoid, polyphenols and flavoproteins which exert their action through scavenging free radicals in vivo system. Hence search for new natural antioxidant is required. Aim of the present study was to determine the antioxidant activity of extracts of different plant materials qualitatively and quantitatively, by in vitro tests. Aqueous and ethanolic extracts were prepared from eight different plant material viz., Phyllanthus emblica (fruit), Tectona grandis (leaves), Solanum nigrum (leaves), Momordica charantia (fruit), Ficus bengalensis (leaves), Asparagus racemosus (roots), Swertia chirata (leaves), Curcuma longa (leaves). Phytochemical analysis of these plant extracts were performed for determination of antioxidant capacity by in vitro qualitative and quantitative tests. In vitro qualitative analysis revealed presence of alkaloid, phenol and flavonoids in aqueous and ethanolic extracts of Tectona grandis followed by Solanum nigrum, Phyllanthus emblica. In vitro quantitative test by FRAP assay revealed highest values in ethanolic extract of P.emblica (6.51±0.08 µ mol of FeSO<sub>4</sub>/mg) followed by aqueous extract of P.emblica (6.16±0.02 µmole of FeSO<sub>4</sub>/mg). Percentage inhibition of Ascorbate iron phospholipid was found highest values in aqueous extracts of T.grandis (55.49±0.25%) followed by P. emblica (54.79±0.17%). Critical analysis of qualitative and quantitative test revealed best antioxidant capacity in aqueous extract of Phyllanthus emblica and Tectona grandis among all 16 extract of different plant materials.

**INTRODUCTION:** The plants and their materials have been utilized for treatment of various diseases in human and animals which is well mentioned in prehistoric literatures like the Rig-Veda, Bible and Quran. India is very rich port with reference to diversity of medicinal plant species and leading country with respect to wealth of recognized knowledge system related to the use of plant species.



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Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments, around 20000 medicinal plants have been recorded but only 7000 - 7500 plants are being used by traditional communities for medicinal purpose <sup>1, 2, 3</sup>. Plants are very rich source of antioxidants. Antioxidant activity in plants is due to nutrients present which shows free radical scavenging capacity *in vivo* system due to presence of phytonutrients such as vitamins (alphatocopherol, ascorbate, carotenoid) or minerals (zinc, selenium) and flavanoid, polyphenols, and flavoproteins <sup>4</sup>.

Further, these plants or species combination of herbs in formulations may act as antioxidants by exerting superoxide scavenging activity or by increasing superoxide dismutase (SOD) activity in various tissue sites <sup>5,6</sup>. In recent year, use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value<sup>7</sup>. Antioxidants derived from fruits, vegetables, spices, and cereals are very effective against free radicals <sup>8</sup>. Antioxidant compounds in their natural formulations are more active than their isolated form <sup>9</sup>.

Although initial research on antioxidants were mostly on isolated pure compounds, recent focus is more on natural formulations <sup>10</sup>. Hence search for new natural antioxidants are needed. Aim of the present study was to determine antioxidant activity of extracts of different plant materials qualitatively and quantitatively, by *in vitro* tests.

#### **MATERIALS AND METHODS:**

**Plants materials:** Following Plant materials viz., *Phyllanthus emblica* (fruit), *Tectona grandis* (leaves), *Solanum nigrum* (leaves), *Momordica charantia* (fruit), *Ficus bengalensis* (leaves), *Asparagus racemosus* (roots), *Swertia chirata* (leaves), *Curcuma longa* (leaves) were selected as per existing scientific literature as well as easy availability of that particular plant in the from adjoining area of Bareilly (U.P). These plant materials were air dried, grinded in mesh form and subjected to aqueous and ethanolic (absolute alcohol) columnar extraction in soxhlet apparatus at temperature of 40-41°C with standard protocol and dried at 41°C temperature.

# In vitro qualitative tests:

- **1. Mayer's Test:** About 0.5g of the plant extracts were dissolved in 5 ml of 1% hydrochloric acid on hot water bath. After cooling, the mixture was filtered and treated with few drops of Mayer's reagent (Potassium mercuric iodide). Formation of yellow cream precipitate indicated the presence of alkaloids <sup>11</sup>.
- **2. Ferric chloride Test:** About 1.0g of plant extracts were dissolved in 10 ml of distilled water and filtered using Whatman No.1 filter paper. The filtrate was treated with few drops of 5% FeCl<sub>3</sub>. Appearance of greenish to black coloration indicates the presence of phenols in extract <sup>11</sup>.

**3. Lead acetate Test:** About 0.5 g of the plant extracts were dissolved in 50% methanol and treated with few drops of 10% lead acetate solution. Formation of yellow color precipitate (ppt) indicated the presence of flavonoids <sup>11</sup>.

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# *In vitro* quantitative tests:

- 1. Ferric Reducing Antioxidant Power Assay (FRAP): The FRAP assay was carried out according to the procedure of Sahgal and coworkers <sup>12</sup>. The FRAP reagent was prepared by mixing acetate buffer (25 ml, 300 mmol/L, pH 3.6), 10 mmol/L TPTZ solution (2.5 ml) in 40 mmol/L HCl and 20 mmol/L FeCl<sub>3</sub> solution (2.5 ml) in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared freshly and warmed to 37°C in a water bath prior to use. Extract samples (150µl) were added to the FRAP reagent (4.5 ml) and the absorbance of the reaction mixture was recorded at 593 nm after 4 min. The assay was carried out in triplicates for accuracy. The standard curve was constructed using FeSO<sub>4</sub> solution (0.1-1.0 mg/ml). The results were expressed as mmol Fe<sup>++</sup>/gm dry weight of plant extracts.
- **2. Ascorbate Iron (III) catalyzed phospholipid peroxidation (AICPP):** The ability of the extracts and the potencies to scavenge hydroxyl radicals was determined by the modified method of Aruoma and coworkers <sup>13</sup>. Goat liver was mixed (1:10) with 10 mM phosphate-buffered saline (PBS, pH 7.4) and sonicated in an ice bath for preparation of the homogenate liposomes. The liposomes (0.2 ml) were added with 0.5 ml of PBS buffer, 0.1 ml of 1 mM FeCl<sub>3</sub> and various volumes (100 μl and 200 μl) of plant extracts and subsequently 0.1 ml of 1 mM ascorbic acid was added. After incubation at 37°C for 60 min, 1 ml of 10% trichloroacetic acid (TCA) was added and centrifuged at 2000 rpm for 10 min at room temperature.

Finally, 1 ml of 0.67% 2-thiobarbituric acid (TBA) in 0.05 M NaOH was added to the supernatant, vortexed and heated in a water bath at 100°C for 20 min. After cooling, 1 ml of distilled water was added and absorbance was recorded at 532 nm. Control containing all reagents except the extracts. The assay was carried out in triplicate for accuracy. The percentage (%) inhibition activity was calculated as:

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% inhibition activity= [(Abs. of control – Abs. of sample)/Abs. of control]  $\times$  100

## **RESULTS:**

The presences of alkaloid, phenols and flavonoids in 16 tested plant extracts are shown in **Table 1** and **Fig.1.** Highly positive (++) alkaloids were noticed in ethanolic extract of *M.charantia*, *F.bengalensis*, *A.racemosus*. The Ethanolic extracts of *T.grandis*, *S.nigrum*, *S.chirata*, and aqueous extracts of *S.chirata*, *T.grandis*, *S.nigrum*, *M.charantia*, *A.racemosus and C.longa* showed positive (+) alkaloid reaction by Mayer's method. Aqueous and ethanolic extract of *P.emblica*, Aqueous extract of *F.bengalensis and* ethanolic extract of *C.longa* showed no reaction. Highly positive (++) phenols

were noticed in ethanolic extracts of *P.emblica*, *T.grandis*, *F.bengalensis*, *C.longa* and aqueous extract of *P.emblica*, *T.grandis* whereas, aqueous extract of *M.charantia*, *F.bengalensis*, *A.racemosus*, *S.chirata*, *C.longa* and ethanolic extracts of *S.nigrum*, *S.chirata* showed positive reaction (+) for phenolic compounds.

Highly positive (++) flavonoids were noticed in aqueous extract of *P.emblica*, *T.grandis*, *S.nigrum*, *M.charantia*, *F.bengalensis*, *S.chirata*, *C.longa and* ethanolic extracts of *P.emblica*, *T.grandis whereas* ethanolic extracts of *S.nigrum*, *M.charantia*, *F.bengalensis*, *A.racemosus*, *C.longa* showed positive(+) reaction for polyphenols.

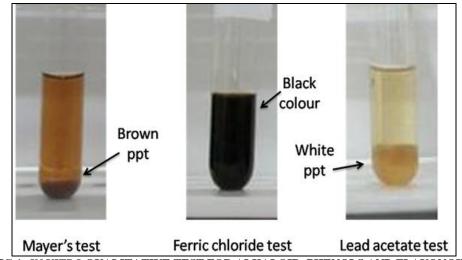


FIG.1: IN VITRO QUALITATIVE TEST FOR ALKALOID, PHENOLS AND FLAVONOIDS

TABLE 1: IN VITRO QUALITATIVE TEST FOR ALCOHOLIC (A) AND ETHANOLIC (E) EXTRACT OF DIFFERENT PLANT MATERIALS

Extracts	Alkaloid	Phenols	Flavonoids
	Mayer's test	FeCl <sub>3</sub> test	Lead acetate test
P.emblica (A)	-	++	++
P.emblica (E)	-	++	++
T.grandis (A)	+	++	++
T.grandis (E)	+	++	++
S.nigrum (A)	+	++	++
S.nigrum (E)	+	+	+
M.charantia (A)	+	+	++
M.charantia (E)	++	-	+
F.bengalensis (A)	-	+	++
F.bengalensis (E)	++	++	+
A.racemosus (A)	+	+	-
A.racemosus (E)	++	-	+
S.chirata (A)	+	+	++
S.chirata (E)	+	+	-
C.longa (A)	+	+	++
C.longa (E)	<u>-</u>	++	+

Highly positive reaction (++), Positive reaction (+) and no reaction (-)

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In vitro quantitative antioxidant activity was determined based on the ability to reduce ferric (III) iron to ferrous (II) iron. The standard curve was drawn in the range of 0.1 mg/ml, 0.2 mg/ml, 0.4 mg/ml, 0.6mg/ml, 0.8 mg/ml and 1.0 mg/ml of ferrous sulphate and the results were expressed as mmol ferrous ion equivalent per gram (mmol  $Fe^{2+}/gm$ ) of sample dry weight (y=0.475x+ 0.085,  $R^2 = 0.996$ ) Fig.2. The aqueous extract Phyllanthus emblica showed highest value  $(6.16\pm0.02 \text{ mmol Fe}^{2+}/\text{gm})$ , followed by Tectona grandis (1.78±0.02 mmol Fe<sup>2+</sup>/gm) and Ficus bengalensis (1.18±0.03 mmol Fe<sup>2+</sup>/gm). Among ethanolic extracts Phyllanthus emblica (6.51±0.08 mmol Fe<sup>2+</sup>/gm), followed by Curcuma longa

(0.90±0.01 mmol Fe<sup>2+</sup>/gm), and *Tectona grandis* (0.88±0.01 mmol Fe<sup>2+</sup>/gm) showed highest FRAP values (**Table 2**). The ability of extracts to scavenge hydroxyl radicals generated by ascorbic – iron III to inhibit the formation of 2-thiobarbituric acid reactive species (TBARS) was tested. The degree of % inhibition of different plant extracts are shown in **Table 2**. The aqueous extract of *Tectona grandis* revealed highest AICPP activity (55±0.25%) followed by *Phyllanthus emblica* (54.79±0.17%). Among the ethanolic extracts of different plants *Curcuma longa* (51.69±0.31%) revealed highest AICPP activity followed by *Phyllanthus emblica* (44.90±0.21%).

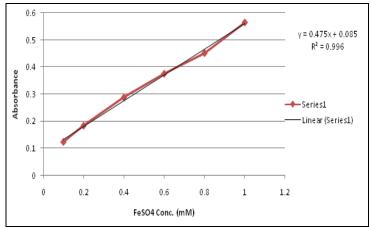


FIG.2: REGRESSION CURVE CONSTRUCTED USING DIFFERENT CONCENTRATIONS OF FERROUS SULPHATE (FeSO<sub>4</sub>) SOLUTION

TABLE 2: IN VITRO QUANTITATIVE ANTIOXIDANT ACTIVITY OF DIFFERENT PLANTS EXTRACT BY FRAP AND AICCP ASSAY

Extracts	Antioxidant Activity by FRAP	Inhibition Activity by AICPP (%)
	(mmol Fe <sup>2+</sup> /gm ext.)	
P. emblica (A)	$6.16\pm0.02$	54.79±0.17
P. emblica (E)	$6.51 \pm 0.08$	44.90±0.21
T. grandis (A)	$1.78 \pm 0.02$	55.49±0.25
T. grandis (E)	$0.88 \pm 0.01$	38.45±0.39
S. nigrum (A)	$0.92 \pm 0.06$	31.53±0.45
S. nigrum (E)	$0.85 \pm 0.01$	$40.68 \pm 0.22$
M. charantia (A)	$0.66 \pm 0.04$	$20.82 \pm 0.08$
M. charantia (E)	$0.43 \pm 0.08$	$10.09 \pm 0.07$
F. bengalensis (A)	$1.18\pm0.03$	51.66±0.20
F. bengalensis (E)	$0.37 \pm 0.08$	11.59±0.18
A. recemosus (A)	$0.61\pm0.03$	$34.79 \pm 0.26$
A. recemosus (E)	$0.45 \pm 0.01$	21.73±0.23
S. chirata (A)	$0.63\pm0.01$	17.74±0.29
S. chirata (E)	$0.40\pm0.01$	$9.09\pm0.46$
C. longa (A)	$0.94 \pm 0.07$	8.12±0.05
C. longa (E)	0.90±0.01	51.69±0.31

**DISCUSSION:** Previous studies reported that *P.emblica* is rich source of vitamin C and tannins,

alkaloids, glycosides, reducing sugars, Resins, flavonoids and phenolic compounds, saponins,

sterol and fixed oil <sup>14, 15</sup>. Present study favours the presence of phenols and flavonoids but not alkaloids by Mayer's test. *Tectona grandis* leaves have been reported to contain carbohydrates, alkaloids, tannins, phenols, flavonoids, sterols, saponins, proteins, calcium, phosphorus, crude fiber and also contain dye <sup>16, 17, 18</sup>. Our studies is accordance with these authors which revealed the presence of alkaloids, phenols and flavonoids in aqueous and ethanolic extract of *T.grandis*. Aqueous and ethanolic extracts of *Solanum nigrum* revealed positive results for alkaloid, phenols, and flavonoids.

Our findings agree with previous study that reviewed, Solanum nigrum plant contains many bioactive constituents such as alkaloids, phenols, tannins, flavonoids and their presence were confirmed by Mayer's test, lead acetate test and ferric chloride test 19. Aqueous extract of fruit of Momordica charantia revealed positive results for alkaloid, polyphenols, and tannins by different test performed while ethanolic extracts showed positive result for alkaloids, flavonoids and negative for phenols. Our study agree with previous findings which revealed presence of alkaloids, steroids, phenolic compounds, flavonoids, anthroquinones and amino acids in fruit extract of Momordica charantia <sup>20</sup>. Aqueous extract of leaves of Ficus bengalensis revealed negative for alkaloids while ethanolic extract revealed the presence of alkaloids. Aqueous and ethanolic extracts of leaves of Ficus bengalensis show presence of phenols and flavonoids. Our finding is agreeing with previous reports which reviewed that aqueous extract of leaves contain sterols, flavonoids, phenol, tannins and saponins in large amount whereas aromatic acids, carbohydrates, triterpenoids, gums, mucilage and volatile oils were totally absent in this plant extract <sup>21</sup>.

Aqueous extract of roots of *Asparagus racemosus* shows the presence of alkaloids, phenols absence of polyphenols while ethanolic extract shows presence of alkaloids, flavonoids and absence of phenols. Phytochemical screening of ethanolic extract of roots of *A.racemosus* confirmed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, tannins, saponins, Steroids, and flavonoids <sup>22</sup>. Aqueous extract of leaves of *Swertia* 

*chirata* revealed the presence of alkaloid, phenols and flavonoids where as ethanolic extract showed presence of alkaloid, phenols and absence of polyphenols. Previous findings reported the presence of flavonoids, xanthones, terpenoids, iridoid and secoiridoid glycosides activity in leaves of Swertia chirata 23. Aqueous extract of leaves of C.longa revealed presence of alkaloid, phenols and flavonoids where as ethanolic extract revealed absence of alkaloid and presence of phenols and flavonoids. Our findings are in accordance with which previous studies revealed the phytochemicals viz.. Alkaloids, Flavonoids. Tannin, Saponins, Cardiac Glycosides and phenol in aqueous extract of Turmeric <sup>24</sup>.

Ferric reducing antioxidant power (FRAP) measures the ability of antioxidants to reduce ferric 2, 4, 6-triperidyl-s-triazine complex to intensively blue colored ferrous complex in acidic medium <sup>25</sup>. Ferric reduction is an indicator of electron donating activity which is an important mechanism of action of phenolic antioxidants <sup>26</sup>. Previous studies reported that P. emblica was able to reduce ferric ion to FeSO4 (7.46±0.56 µ mole of FeSO4/mg) which is about 0.89 fold compared to Trolox (8.35±0.16 μmole of FeSO4 /mg). Our study agrees with above reports <sup>27</sup>. Aqueous extract of leaves of F. bengalensis revealed 1.18±0.03 mmol Fe<sup>2+</sup>/gm FRAP value. Our findings disagree with previous reports <sup>28</sup>.

Phospholipid liposomes undergo rapid non-enzymatic peroxidation when incubated in the presence of FeCl<sub>3</sub> and ascorbic acid. Present investigation revealed highest inhibition % by the aqueous extract of *Tectona grandis* ( $55\pm0.25\%$ ) followed by *Phyllanthus emblica* ( $54.79\pm0.17\%$ ), ethanolic extracts *Curcuma longa* ( $51.69\pm0.31\%$ ). Earlier reports revealed that fresh bark of Tectona grandis showed  $53.67\pm0.31$  % inhibition  $200\mu g/ml$  of ascorbic acid <sup>29</sup>. Several studies asserted that *P. emblica* contains a high % of ascorbic acid of the fruit which accounts for 45-70% of the antioxidant activity <sup>30,31</sup>.

**CONCLUSION:** The qualitative and quantitative *in vitro* phytochemical analysis revealed maximum antioxidant properties in aqueous extract of

Phyllanthus emblica and Tectona grandis among all 16 extracts of different plant materials.

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