EVALUATION OF ANTIOXIDANT ACTIVITY OF IN VITRO PROPAGATED PLANTS (L.)

P.P.More[1, 2], B.Y.Patil[2], G.S.Gond[1], and J.A. Kulkrni
1. S.M.D.M. Collage Kalamb Dist. Osmanabad
2. Department of Biochemistry, Guru Nanak Science College, Ballarpur Dist. Chandrapur.
3. Department of Biotechnology, Dr. Babasaheb Ambedkar Marathwada University, sub-campus Osmanabad.

Abstract: ***The present research work is screening of antioxidant activity by two methods DPPH (2-2 Diphenyl 1-picryl hydroxyl) & Reducing power assay through the in vitro propagated plants i.e. Bahunia Purpurea, Syzygium Cumini, Cassia Tora . It includes various solvent (Organic,Aqueous) & ethyl acetate ,Methanol extract. It has been compared with ascorbic acid in reducing power assay. The methanol extract showed significant results by the Reducing power assay & DPPH assay, but methanol extract shown medium activity of Bahunia Purpurea, Syzygium Cumini, Cassia Tora but highest activity shown in aqueous extract of Bahunia Purpurea & Syzygium Cumini by Reducing power assay whereas ethyl acetate extract of Cassia Tora shown medium activity .***
In the DPPH, assay aqueous extract of Bahunia Purpurea, shown highest activity than aqueous extract of Syzygium Cumini as it shown medium acitivity. Further that,highest activity present in methanol extract of Syzygium Cumini whereas medium activity present in methanol extract of Bahunia Purpurea and lowest activity shown by methanol extract of Cassia Tora .

IndexTerms : DPPH,Reducing power assay, Bahunia Purpurea, Syzygium Cumini, Cassia Tora ,antioxidant activity.

Introduction:

There are various natural antioxidant compounds present in medicinal plant. The Aim of Scientific studies of medicinal plants work to cure disease in their chemical constitute which playing an important role in physiological systems .Antioxidant have been used in food Industry to prolong the shelf life of foods especially those rich in polyunsaturated. These are secondary metabolism of plants **(Walton & Brown 1999)** like flavonoids, polyphenols, tannins & nitric acid, scavengers of free radicals, peroxidase &hydrogen peroxidase .Thus inhibits the oxidative mechanism that lead to degenerative disease.**(Lekha K. Nair & Maleeka Begum 2013)** Reactive oxygen species (ROS) are generated as a byproduct of biological reaction & from exogenous factors. Some of them are important in cell metabolism including energy production, phagocytosis & intercellular signaling **(Ottolenghi 1959).** Ionizing radiation, chemical reaction, ultraviolet light, Sun-light create by Reactive oxygen species (ROS) & Result as DNA Damage, Carcinogenesis & various degenerative disorders such as cardiovascular diseases aging & neuro- degenerative diseases**.(Gyamfi 1999,Sawa 1994, Noda 1997).** B. Purpurea is flowering plant native of south china in the family of Fabaceae, is small to medium sized deciduous tree.**(Chaluvadi MR, Krishna DR, Chinedu P.A.)**B. Purpurea is also reported to be used in treatment of Jaundice, Leprosy, Cough, Pain, fever, Ulcers, stomach , Cancer,rhenatism, Convulsions, delinium & Septicaemia **(Chopra 1956 ; Asolkar 2000, Parrota 2001; Kirthikar & Basu 2001, Janardhanan 2003**) as well as treatment of diarrhea, boils & abscesses**,(Kirthikar & Basu 2001).** They are reported to exhibit various pharmacological activities such as CNS activity, Cardiotonic activity, Lipid Lowering activity, anti-oxidant activity, hepato-protective activity, hypoglycemic activity. **(Rajanarayana K. Reddy MS)** The Species of syzygium cumini belong to myrataceae family is a worldwide medicinal plant traditionally used in herbal medicines due to its valuable properties against cardio metabolic disorders which include anti hyperglycemic, anti-inflammatory, cardio protective & antioxidant activities (Vinicyus Teles chages,Lucas Martins franca).S.Cumini tree native from India widely cultivated in many countries in Asia, Africa as Jamun also known black Jamun in Europ & South America**.(Srivastva & Chandra 2013 ,Correa 1974.)**. Dietary antioxidant include ascorbate, tocopherols, Carotenoids & bioactive plant phenols including flavonids, phenolic, acids & volatile compound for preventing oxidation of bimolecules.(Elizabeth Margaret) In food system oxidation of lipid resulting in rancidity deterioration of sensory properties of food pose a major problem for both consumers & manufactures. One more important role is preventing undesirable flavor & maintaining the nutritional quality of the food**.(shahidi & wasundara 1992).** The plant possesses acetyl olenolic acid, triterpenoids, ellagic acid, Isoquercitin, quercetin & myricetin in different concentrations **(Rastogi R & Mehrotra BN 1990)**. Cassia tora belongs to caesalpiniaceae family mostly used for treatment of ulcer, in India & as traditional medicine in Africa. The phytochemical Screening of leaf showed the presence of polyphenols which prompted research to evaluate its antioxidant & antiproliferative potential, Cassia tora is used in treatment of various disease like aging, asthma,diabetes ,Eye disease , & the origin is deleterious free radical reaction**. Chan M.J. & Peria L.M.(2001)**

Antioxidant present in fruits & vegetables are the main factors to decrease the incidence of chronic disease **(liu2003)** free radicals are produced either from normal metabolic processes in the body or external sources such as exposure to radiation etc**.(lobo 2010).** According to antioxidant defense system, it may increase the oxidative burden & damage macromolecules.**(Jaikumar 2010). Uddin (2008**) evaluated that methanol & aqueous extract of the dries aerial part of cassia tora were subjected to the potential antioxidant activity Methanol extract of the cassia tora possess strong antioxidant activity & aqueous extract showed medium antioxidant activity. The plant cassia tora showed pharmacological activity.Anti-inflammatory **(Maity 1998)** Antinoceptive Activity. **(Chidum 2002)** Antishigellosis Activity.**(Awal 2004)** Hypolipidemic Activity **(Umesh 2004)**Estrogenic Activity **(Nakamuru 2007)** Antioxidant activity **(uddin 2008)** Antimicrobial activity **(murkhero 2000)** Anthelminitic Activity **(Derore 2009)** Antinociptive Activity **(chidum 2002)** Hypolipdemic Activity **(Umesh 2004)** Hepatoprotective Activity .Although the objective of current work was studied of antioxidant activity by in vitro propagated plants.

Method and Material:

Collection of Plants material: Cassia Tora, Syzygium Cumini, Bahunia Purpurea

Extraction:

Organic Solvent Extraction: Ethyl Acetate, Methanol.

(Lekha K. Nair Maleeka Begum 2013., Murugan M. 2011., S. Shyamala Gowri and K. Vasantha 2010,)

Aqueous Solvent Extract: (Murugan M. 2011. S. Shyamala Gowri and K. Vasantha 2010,) **Antioxidant Assay:**

Reducing Power Assay: (Oyaizu et. all 1986)
DPPH Assay :(lekha K Nair, Maleeka Begum 2013)

Methods:

Organic Extract preparation: Crush powder (10%) of disinfected condition by propagated plant (through plant tissue culture) was mixed with 10 ml organic solvent (Methanol, Ethyl Acetate, Hexane,) & sealed with Parafilm. The mixture was kept in shaker for overnight or 24 hours at 100 rpm, then mixture was placed at centrifugation 3000 rpm for 15 minutes. The solution was filtered by muslin cloth & re-filtered by whatman filter paper nine.

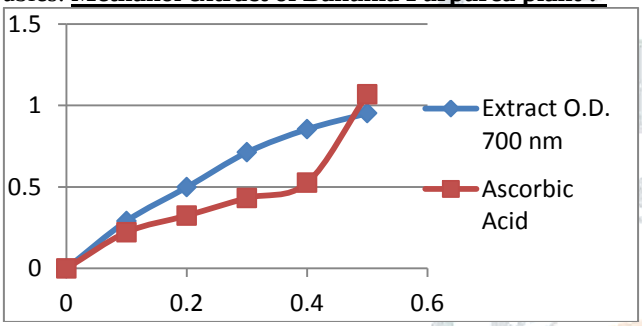
Antioxidant Activity:

Reducing Power Assay:-Take 1 ml of different concentration of sample (Extract sample) 4.5, 9.0, 13.5,18.0, 22.5, 27.0 mg/ml ,were mixed with 2.5 ml potassium ferrocynide (1%) kept for incubation of 50° C water bath for 20 min. After Incubation 2.5 ml TCA (10 %) was added to terminate the reaction. Pipette out 2.5 ml upper portion of the solution was mixed with 2.5 ml D/W. Then 0.5 ml $fecl_3$ (0.1 %) was added, make up volume in each tube up to 3 ml. The reaction mixture was left for 10 minute at normal room temperature and absorbance was measured at 700 nm against an appropriate blank solution. A higher absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used as positive control. The fe3 + reducing power of the extract was followed by (Oyaizu et al… 1986.)

DPPH Assay: This was assayed as described by Elizabeth & Rao (1990) the reaction mixture contained methanol 50 ml. DPPH (Diphenyl 2-picryl hydrazyl radical) 0.3 mM, 1ml of 0.3 mM DPPH in methanol was added to 100µl of compound with concentration ranging from 20 µg to 100µg. DPPH solution with methanol was used as a positive control and methanol alone acted as a blank. When DPPH reacts with antioxidants in the sample, it was reduced and color changed from deep violet to light yellow. This was measured at 517 nm

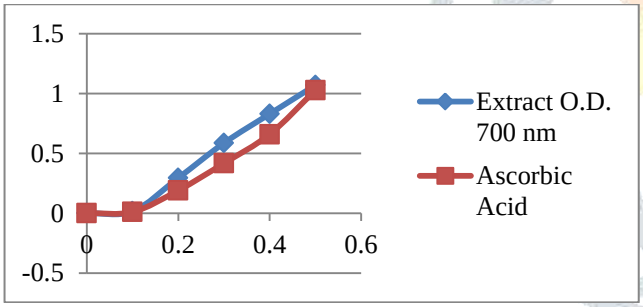
Reducing power assay:-

Tables: **Methanol extract of Bahunia Purpurea plant :-**



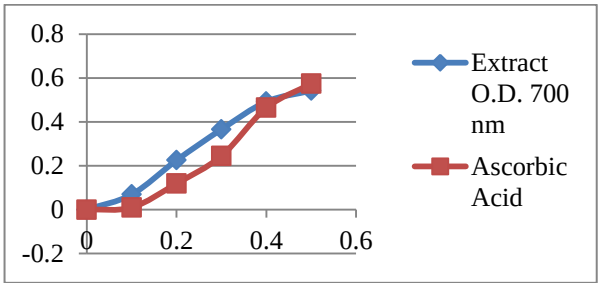
Sr.No.	Plant Extract	Extract O.D. 700 nm	Ascorbic Acid
1	0	0	0
2	0.1	0.291	0.222
3	0.2	0.498	0.324
4	0.3	0.712	0.432
5	0.4	0.854	0.526
6	0.5	0.952	1.069

Aqueous Extract of Bahunia Purpurea Plant



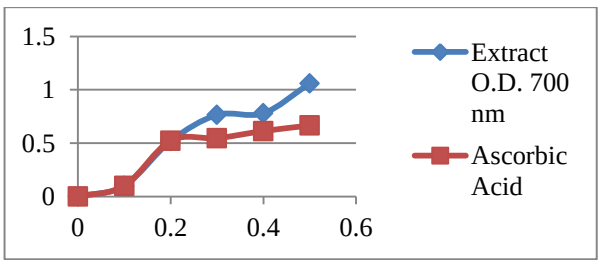
Sr.No.	Plant Extract	Extract O.D. 700 nm	Ascorbic Acid
1	0	0	0
2	0.1	0.015	0.010
3	0.2	0.294	0.190
4	0.3	0.586	0.417
5	0.4	0.829	0.659
6	0.5	1.069	1.027

Methanol Extract of Syzygium Cumini



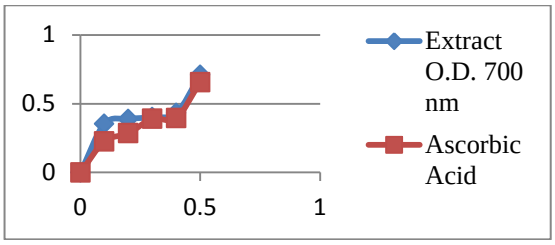
Sr.No.	Plant Extract	Extract O.D. 700 nm	Ascorbic Acid
1	0	0	0
2	0.1	0.069	0.010
3	0.2	0.225	0.119
4	0.3	0.366	0.245
5	0.4	0.492	0.464
6	0.5	0.542	0.574

Ethyl acetate Extract of Syzygium Cumini :



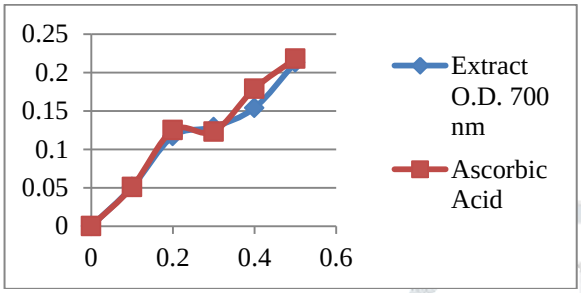
Sr.No.	Plant Extract	Extract O.D. 700 nm	Ascorbic Acid
1	0	0	0
2	0.1	0.10	0.10
3	0.2	0.520	0.523
4	0.3	0.764	0.547
5	0.4	0.780	0.613
6	0.5	1.060	0.665

Aqueous Extract of Syzygium Cumini Plant



Sr.No.	Plant Extract	Extract O.D. 700 nm	Ascorbic Acid
1	0	0	0
2	0.1	0.354	0.226
3	0.2	0.389	0.285
4	0.3	0.402	0.390
5	0.4	0.432	0.396
6	0.5	0.709	0.656

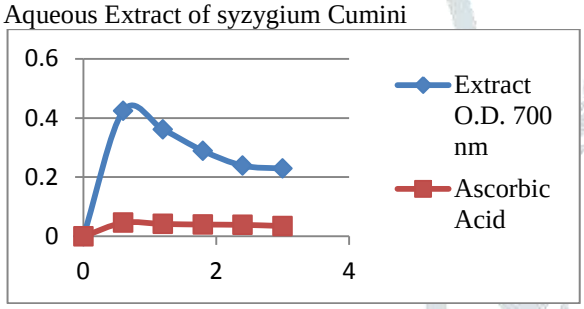
Methanolic Extract of Cassia Tora



Sr.No.	Plant Extract	Extract O.D. 700 nm	Ascorbic Acid
1	0	0	0
2	0.1	0.051	0.051
3	0.2	0.117	0.125
4	0.3	0.129	0.123
5	0.4	0.154	0.179
6	0.5	0.213	0.218

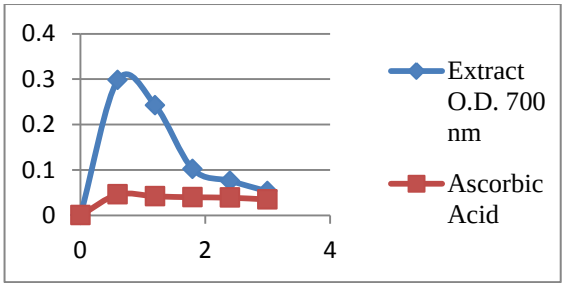
DPPH Assay

Aqueous Extract of syzygium Cumini



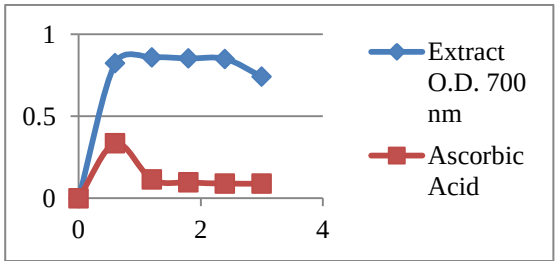
Sr.no	Plant Extract	Extract O.D. 700 nm	Ascorbic Acid
1	0	0	0
2	0.6	0.424	0.046
3	1.2	0.361	0.042
4	1.8	0.289	0.04
5	2.4	0.239	0.039
6	3	0.229	0.035

Methanol extract of syzygium cumini



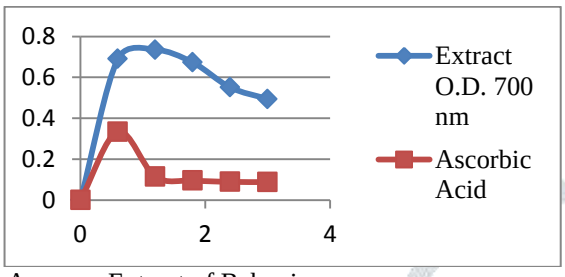
Sr.no	Plant Extract	Extract O.D. 700 nm	Ascorbic Acid
1	0	0	0
2	0.6	0.298	0.046
3	1.2	0.242	0.042
4	1.8	0.102	0.04
5	2.4	0.076	0.039
6	3	0.053	0.035

Methanol extract of Cassia tora



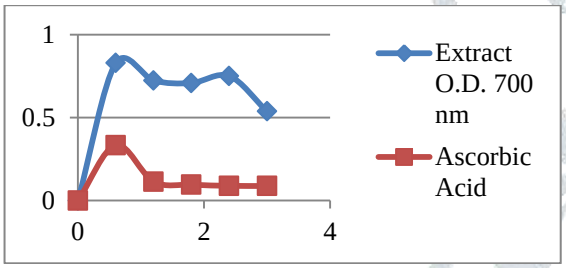
Sr.no	Plant Extract	Extract O.D. 700 nm	Ascorbic Acid
1	0	0	0
2	0.6	0.823	0.334
3	1.2	0.858	0.114
4	1.8	0.852	0.096
5	2.4	0.849	0.089
6	3	0.741	0.088

Methanol extract of bahunia purpurea



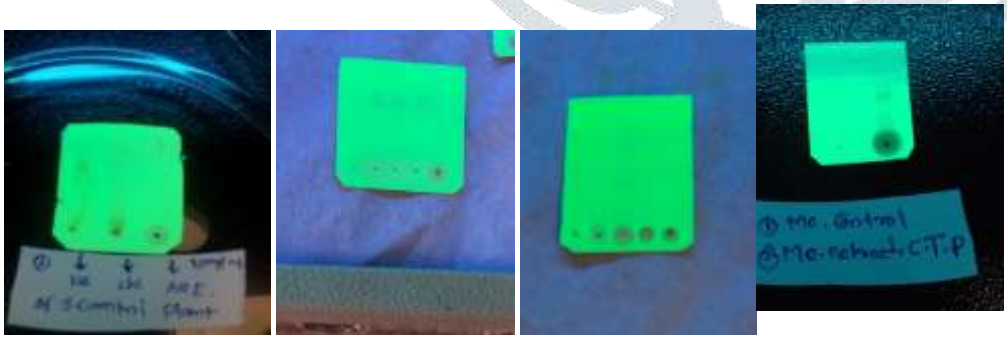
Sr.no	Plant Extract	Extract O.D. 700 nm	Ascorbic Acid
1	0	0	0
2	0.6	0.690	0.334
3	1.2	0.735	0.114
4	1.8	0.673	0.096
5	2.4	0.551	0.089
6	3	0.494	0.088

Aqueous Extract of Bahunia purpurea



Sr.no	Plant Extract	Extract O.D. 700 nm	Ascorbic Acid
1	0	0	0
2	0.6	0.828	0.334
3	1.2	0.723	0.114
4	1.8	0.708	0.096
5	2.4	0.75	0.089
6	3	0.537	0.088

Thin layer Chromatography: TLC technique specially used for separation of non-volatile compounds from the plant extracts. The plant extracts (Hexane, Chloroform, ethyl acetate, methanol) sample applied in silicon plate. This is known as mobile phase. After the experiment the spot are visualized by using ultra-violet light on to the sheet. There is one more method used for visualized spot on a sheet.Which is called as chemical process in that include anisaldehyde and sulfuric acid.



Photos: *a) Syzygium cumini. b&c) Bahunia purpurea d) Cassia tora.(L.)*

Results and Discussion: Determination of Antioxidant activity of *Bahunia Purpurea*, *Syzygium Cumini and Cassia Tora* plants with methanol, ethyl acetate and aqueous extract by DPPH assay & reducing power assay. Antioxidant activity by reducing power assay of *Bahunia Purpurea* through methanol extract given significant result; but highest activity shown in aqueous extract. In *Syzygium Cumini* - Methanol, Ethyl acetate & aqueous extract given good results; where highest results shown in Methanol, then medium activity by Ethyl acetate extract and lowest activity in aqueous extract. Whereas *Cassia Tora plant* shown medium activity by the methanol extract. According to above assay *Bahunia Purpurea*, *Syzygium Cumini and Cassia Tora have* shown good response in antioxidant activities.

Further, antioxidant activities analyze by DPPH assay of *Bahunia Purpurea*, with methanol extract & aqueous extract; in that case methanol extract given significant results than aqueous extract. Plant of *Syzygium Cumini*, Ethyl acetate extract shown lower activity than the methanol extract activity, last but not least methanol extract of cassia tora shown lower activity by DPPH assay.

Reducing power assay values compared with positive control i.e. ascorbic acid was used to evaluate the antioxidant capacity of various organic & aqueous solvent in the present work & efficiently absorb UV light 700 nm. The RPA values of in vitro propagated *Syzygium Cumini* plants were highest than *Bahunia Purpurea & Cassia Tora*.

In DPPH assay, DPPH is clarifying antioxidant compounds the purple color of DPPH changed to yellow color. The more yellowish colour of DPPH observed the greater antioxidant activity of the compound tested. The results methanol extract of *Syzygium Cumini* & aqueous extract of *Bahunia Purpurea,* shown highest activity than methanol extract of *Bahunia Purpurea,*& aqueous extract of *Syzygium Cumini* .similar to that lowest activity shown in methanol extract of *Cassia Tora*.& efficiently absorb UV light 517 nm.
In Thin layer chromatography result observed from *syzygium cumini* plant extract i.e. Hexane, Chloroform and aqueous but best result observed of hexane, chloroform than aqueous extract. Methanol extract o*f cassia tora* plant showed a single band, compare with methanol. In *Bahunia purpurea* used organic solvent i.e. Hexane, chloroform, ethyl acetate, Acetone and aqueous extract, among them didn't showed any band in aqueous extracts. whereas good band observed in organic solvent.

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