Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(4): 1477-1481
Received: 05-05-2018
Accepted: 10-06-2018

Swati Goswami
Department of Industrial Microbiology, Jacob Institute of Biotechnology and Bioengineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Reena Jain
Associate Professor, Boston College for Professional Studies, Sirol Main Road, Gwalior, Madhya Pradesh, India

Harison Masih
Assistant Professor, Department of Industrial Microbiology, Jacob Institute of Biotechnology and Bioengineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Correspondence
Harison Masih
Assistant Professor, Department of Industrial Microbiology, Jacob Institute of Biotechnology and Bioengineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Comparative evaluation of *In vitro* antioxidant analysis of various leaves extracts for selected medicinal plants

Swati Goswami, Reena Jain and Harison Masih

Abstract
Aegle marmelos, Cocculus hirustus, Grewia asiatica and Populus deltoides sp. leaves extracts were evaluated for their antioxidant potential with various *in vitro* tests including nitric oxide radical scavenging assay, reducing power assay and total antioxidant property by phosphomolybdate method. It was found that nitric oxide radical scavenging property was highest for *A. marmelos* leaves acetone extract (AMLAE) (57.65 ±0.46 µg/mL) while the highest reducing power ability was exhibited by *A. marmelos* leaves aqueous hot extract (AMLAqHotE) (871.09 ±14.52 µg/mL) and *P. deltoides* leaves acetone extract (PLAE) (61.78 ±1.74 µg/mL) demonstrated highest total antioxidant property as estimated by phosphomolybdate method. This conclude that the leaves extracts of these plants have strong potential for scavenging free radicals thus can be used for synthesis of potent antioxidant drug component for various diseases caused due to oxidative stress like cancer, aging, inflammation, cardiovascular diseases, neurodegenerative diseases, and liver injury etc.

Keywords: free radicals, antioxidants, *Aegle marmelos, Cocculus hirustus, Grewia asiatica, Populus deltoides*

Introduction
Oxygen is an essential element with double edged properties as obligatory for life and eventuates to the destruction in the cell by oxidative events (Brieger *et.al*, 2012) [19]. On one side oxidation of fats, proteins and carbohydrates acts as source of energy for the body and on other side are the progenitor of free radicals. Free radicals detrimental effects were unveiled in last decade. These are produced in the body during the normal metabolic process along with wastes and toxins. Deluge of free radicals are responsible for abnormalities in biological molecules including protein enzymes, DNA and RNA along with tissue injuries (Partap S., 2012) [20]. Free radicals lead to various diseases including cancer, aging, rheumatoid arthritis etc.

Antioxidants are molecules of plant genesis or artificially synthesized. It acts either directly by reacting with free radicals or indirectly by inhibiting the expression or activity of enzymes produced in response to free radical. Antioxidants are also known to accelerate the expression of cellular antioxidant enzymes thus decreasing the level of oxidative stress (Lü a. *et. al*, 2010). Phytochemicals are natural antioxidants that are capable enough to stop free radical chain reactions (Saxena, *et. al*, 2013) [21]. These have various benefits to their name, being safe as well as acting as protection cover over the body from any type of injury. Phytoconstituents are compatible to body physiology as compared to synthetic antioxidant compounds that are reported to have mutagenic and toxic to body thus drifting the focus towards naturally originated antioxidant (Pham-Huy *et. al*, 2008) [22]. Phytomedicins and phytochemicals are the vanguard of antioxidant synthesis.

Aegle marmelos (Linn.) commonly known by the name "bail" has various hidden pharmacological properties being antidiarroheal, antibacterial, antiulcer, antifungal, antiviral, anticancer, anti- inflammatory, analgesic, antifertility, antipyretic antihyperglycemic, antithyroid, antidyslipidemic, as well as antioxidant effects. (Sabu *et. al*., 2009; Sudharameshwari, 2007) [2].

Cocculus hirsutus (Linn.) Diels, known as Chilahinta has vast medicinal properties and used in the treatment of polyuria, dysuria, eczema, rheumatoid arthritis, abdominal disorders, piles, fevers, disorders of blood syphilis, aphrodisiac and also possess cardiotonic, antimicrobial, diuretic, hyperglycaemic, epileptic, laxative and activity (Ramalingam R, 2012; Madhavan V, 2010; Nayak S. 2003; Ganapaty S, 2002; Satyanarayana K, 2001) [18, 6, 3, 4, 5].

Grewia asiatica Linn member of Tiliaceae family, known as 'Phaalsa', has reveled medicinal properties thus used as hypotensive, spasmolytic, antifebrile, cardio-protective, anti-fertile,

analgesic, anti-oxidant, anti-biotic, anti-diphtheria, micronutrient, radioprotective, anti-arthritic, etc. (Joshi *et. al*, 2013; Goswami *et. al*, 2018) [7, 23].

Populus sp**.** belongs to family *Salicaceae,* commonly known as Poplar. Traditionally its bark and balsam from leaf bud is used for cold. Bark is depurative and leaf bud is antiseptic, anti-inflammatory. The bud exudate contains dimethylcaffeic acid, which was found active against herpes simplex virus type I. The bark of all *Populus* species contains phenolic glycosides, salicin and populin (salicin benzoate) (Merghache *et. al*, 2016; Picard *et al*., 1994) [8, 24]. Traditionally decoction of rotten leaves was used as an herbal bath for general body pain (Duke, J.A. 1983c) [25].

Having so many health benefits, as reported in Ayurveda and Unani medicine, our research focus on comparison and evaluation of antioxidant potential of leaves extracts of *Aegle marmelos, Cocculus hirustus, Grewia asiatica and Populus* sp. prepared in different solvents using nitric oxide radical (NO) scavenging assay, reducing power (RP) ability and total antioxidant potential using phosphomolybdate (PM) assay.

Material and Methods

Plant Material

The fresh and healthy leaves of *A. marmelos, C. hirustus, G. asiatica* plants were collected from various sites of Gwalior (M.P.) *and Populus* sp. leaves from Allahabad in 2015-17. Plant materials were identified with the help of local community as well as using the mobile based software application "Picture This- Plant identification" offered by Hangzhou Glority software Ltd. and were authenticated by Dr. Harison Masih, Assistant professor at Department of Industrial Microbiology, JIBB, SHUATS, Allahabad.

Preparation of plant extracts using various organic solvents

Freshly collected plant parts were surface sterilized using 0.1% $HgCl_2$ followed by repeated washing with sterile phosphate buffer saline (pH 7.2) and distilled water. Plant parts were than dried at 50ºC using electric drier and crushed with the aid of a mechanical grinder to powdered form. These powdered plant parts were used to prepare different extracts as described below. Leaf extracts of *A. marmelos, C. hirustus, G. asiatica and Populus* sp. were prepared in aqueous as well as organic solvents like acetone, ethyl acetate, methanol and petroleum ether.

Nitric oxide radical scavenging assay

Leaf extracts of the plants were taken at the concentration of 10mg/mL and mixed with 10 mM sodium nitroprusside in 0.025 M phosphate buffer saline (pH 7.4).The reaction mixture was made to 3mL final volume and incubated at 25°C for 150 min. Negative control experiment without the sample was processed in same manner. Thereafter, 0.5 mL of incubation solution was diluted with 0.5 mL Griess' reagent in separate test tube and allowed to stand for 30 min. Absorbance of chromophore formed was read at 546nm during diazotisation of nitrite with sulphanilamide and subsequent coupling with naphthyethylene diamine dihydrochloride. Curcumin was taken as positive control and the experiment was performed in triplicate (Sreejayan and Rao, 1997; Yildirim *et. al*, 2001) [9, 10].

Reducing power ability

Reducing power ability was measured by mixing 1.0 mL extract prepared with distilled water to 0.2 M phosphate buffer (pH 6.6) and 1 % potassium ferricyanide followed by incubation at 50°C for 30 minutes. To this mixture, trichloroacetic acid (10%) was added and centrifuged for 10 min at 3000g. From this mixture, 2.5 mL of supernatant was taken and diluted with equal volume of water followed by shaking with 0.1% ferric chloride. The absorbance was measured at 700 nm using UV-Visible spectrophotometer. The reference solution was prepared as described above, except sample. Increased absorbance of the reaction mixture indicates increased reducing power. All experiments were done in triplicate using ascorbic acid as positive control (Fejes *et.al*, 2000) [11].

Total antioxidant property by Phosphomolybdate method

The total antioxidant capacity for leaf extracts was determined with phosphomolybdenum taking α-tocopherol as the standard. An aliquot of 0.1 mL of plant leaf extract (100 µg) solution was combined with 1.0 mL of reagent containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The tubes were incubated in boiling water bath at 95ºC for 90 min. Samples were allowed to stand for cooling at room temperature. The absorbance of samples were measured at 695 nm in UV- Visible spectrophotometer. The total antioxidant capacity was expressed as equivalents of α-tocopherol (Jayaprakash *et. al*, 2002; Prieto *et. al*, 1999) [12, 17].

Statistical analysis

Graph Pad Prism 5 software (H.J. Motulsky, Prism5 Statistics Guide, Graph Pad Software Inc., San Diego, CA, USA, www.Graph Pad.com) was used was used to calculate the IC_{50} values and experiments were performed in triplicate. Two tailed T-test was applied to investigate the differences among ascorbic acid equivalent antioxidant concentration (AEAC) and α-tochopherol equivalent values of leaves extract in different solvents. All the assays findings were subjected to the Student t test ($p < 0.01$) to determine their significance.

Results and Discussion

Free radicals are archived for playing a twin role in our body as both deleterious and beneficial species. Decrease in concentration of antioxidants and increase in concentration of free radical results in oxidative stress. This is a harmful process that can mediates damage to cell structures, including proteins, lipids, DNA and RNA, thus genesis for number of diseases. A range of synthetic medicine engaged in the treatment of different diseases also competent enough to generate free radicals in body which may lead to another disease. The plant origin components are abundant of antioxidants, capable to end free radical reactions and prevent our body from oxidative damage. The antioxidants are keys to vide range of metabolic disorders caused by reactive species or free radicals which were critically evaluated among various leaves extracts of *A. marmelos, C. hirsutus, G. asiatica* and *Populus* sp. using various antioxidant assays including nitric oxide scavenging method, phosphomolybdate method and reducing power ability of these extracts as compiled in table 1. The values were represented as AEAC for both nitric oxide scavenging assay and reducing power ability. The α-tochopherol was used as standard for phosphomolybdate method. Phagocytes and endothelia cells produce nitric oxide free radical in order to activate series of reaction resulting in formation of OH radicals via peroxynitrite as intermediate product (Reshma *et. al*, 2014) [13]. In the present study, NO radical scavenging potential was estimated for leaves extract

(in different solvent system) of plants, the results of which are graphically presented in fig 1. Significant reduction of NO radical mediated by AMLAE (57.65 ± 0.46 µg/mL) was observed, followed by AMLME (54.86 ± 0.89 µg/mL) and PLAE (51.02 ± 0.52 µg/mL). Their IC_{50} value was calculated as 770 µg/mL, 809 µg /mL and 870 µg /mL for AMLAE, AMLME and PLAE respectively. Nitric oxide radical scavenging activity for *A. marmelos* leaves water extract was reported 29.06 µg/mL by Reshma *et al*, (2014) [13]. Panda *et al*, in 2011 reported 92.4% of NO reduction by ethanol leaves extracts of *C. hirsutus*. Purwar S., 2015 [15], reported NO reduction values of methanol, petroleum ether and aqueous extracts of *G. asiatica* as 56.88 ± 16, 22.12 ± 2.65 and 152.75 ± 5.76 µg/mL respectively. The presence of reductones serves the purpose as having antioxidant activity which terminates the free radical chain reaction by donating a hydrogen atom to it. Among the various extracts under study for its reducing power activity, the highest potential was obtained for AMLAqHotE (871.09 ± 14.52 µg/mL) followed by AMLEtAE (77.91 ± 4.36 µg/mL) and CHLEtAE (61.91 ± 3.90 µg/mL) with their IC_{50} values as 1073 µg /mL, 481 µg/mL and 6367 µg/mL respectively. The results are presented in Table 1 and the same has been graphically presented in fig 2. Kumar *et. al.* (2016) [16] reported the IC_{50} value, determined by the reducing power ability method for methanol extract of *A. marmelos* to be 475.42 ± 25.95 µg /mL while our findings for the same extract is 3335 µg /mL. For water extract of leaves, Reshma *et. al,* (2014) [13] reported the IC_{50} value for *A. marmelos* to be 17.84 µg/mL. All the leaves extracts were also found to reduce molybdenum VI into green colored phosphomolybdenum V complex as determined through phosphomolybdenum method, the readings for the same are presented in Table 1 and fig 3. This was exploited to compare total antioxidant potential of various plant leaves extracts. Scientific studies on different leaves extract *Populus* sp. are scarce. The present study is an attempt to report the antioxidant properties of leaves extracts of *Populus deltoides* which has not been reported in recent past. Ethanolic leaves extract of *P. deltoides* (PLAE) showed the highest potential (61.78 ±1.74 µg/mL) followed by PLAqCold E (54.44 ±1.64 µg/mL) and GALAqHotE (57.76 ±1.69 µg/mL) along with their IC_{50} value as 273 µg mL, 310 µg /mL and 292 µg /mL respectively.

Conclusion

Hence, on the basis of the findings of present investigation, it can be suggested that leaves extracts of these plant contains phytochemicals that are having high antioxidant potential. Thus these can be used in antioxidant-based drugs/formulations for abstention and regimen of diseases like atherosclerosis, diabetes, stroke, Parkinson's disease, Alzheimer's disease (AD), cancer, etc. Free radical theory has greatly increased the interest in the role of antioxidants in preventing many human diseases, including, atherosclerosis, cancer, stroke, neurodegeneration and rheumatoid arthritis diabetes.

Table 1: Different antioxidant assays are presented for various solvent extracts.

Plant extract	NO AEAC (µg/mL)	Plant extract IC_{50} (µg/mL)	RP AEAC (µg/mL)	Plant extract IC_{50} (µg/mL)	PM α-tocopherol equivalent (µg/mL)	Plant extract IC_{50} (µg /mL)
AMLAE	57.65 ±0.46	771	59.42±3.81	683	48.64 ±1.55	346
AMLAqCold E	45.59 ±0.41	974	43.41±3.27	2815	38.51 ±1.38	438
AMLAqHotE	47.98 ±0.50	926	871.09±14.52	815	44.38 ±1.48	380
AMLEtAE	33.33 ±0.70	1333	77.91 ±4.36	4813	49.19 ±1.56	343
AMLME	54.86 ±0.89	810	51.79±3.57	3335	45.96 ±1.50	367
AMLPE	27.45 ±0.82	1619	18.34±2.14	6049	0.32 ±0.13	5189.0
CHLAE	20.80 ±0.55	2136	46.85±3.39	4717	15.11 ±0.86	1117
CHLAqCold E	36.89 ±0.29	1204	13.96±1.87	5420	16.03 ±0.89	1052
CHLAqHotE	19.39 ±17.24	2701	28.86±2.67	1215.6	2.30 ±0.34	7383
CHLEtAE	26.64 ±0.62	1668	61.91±3.90	6367.3	4.76 ±0.48	3559
CHLME	20.31 ±0.60	2188	8.78±1.49	6956	0.21 ±0.10	8263.5
CHLPE	11.82 ±0.45	3760	0.82±0.52	4131.1	0.39 ±0.14	4351.1
GALAE	6.19 ±0.21	7179	35.39 ±2.95	4631	38.51 ±1.38	438
GALAqCold E	40.48 ±0.92	1098	20.89±2.28	7043	40.81 ±1.42	413
GALAqHotE	32.72±0.89	1358	37.71±3.05	1459.4	57.76 ±1.69	292
GALEtAE	1.01 ±0.06	4390.9	27.84±2.62	5374	31.00 ±1.24	544
GALME	25.84 ±0.67	1720	5.51±1.20	1220	43.35 ±1.46	389
GALPE	36.60 ±0.44	1214	35.39±2.95	1902.6	0.01 ±0.02	4131.7
PLAE	51.02 ±0.52	871	16.99±2.06	6680	61.78 ±1.74	273
PLAqCold E	49.65 ±0.51	895	5.82±1.23	4030.8	54.44 ±1.64	310
PLAqHotE	34.59 ±0.43	1284	36.16±2.98	8.542	39.00 ±1.38	432
PLEtAE	39.11±0.45	1136	4.35±1.07	28.366	19.55 ±0.98	863
PLME	37.26 ±0.51	1193	13.01±1.81	13.730	10.14 ±0.71	1666
PLPE	38.14 ±0.37	1165	8.78±1.49	20.966	0.92 ±0.22	1922.1

Data is represented as mean ± standard deviation, data was analyzed using two tailed T test at α = 95%

Note: Abbreviations used in table are as follow: NO=Nitric oxide scavenging assay; AEAC= Ascorbic acid equivalent antioxidant concentration; IC_{50}=Inhibitory concentration; RP=Reducing power ability; PM= Total antioxidant activity using Phosphomolybdate method; AMLAE=*A. marmelos* leaves acetone extract; AMLAqCold E= *A. marmelos* leaves aqueous cold extract; AMLAqHotE= *A. marmelos* leaves aqueous hot extract; AMLEtAE= *A. marmelos* leaves ethyl acetate extract; AMLME= *A. marmelos* leaves methanol extract; AMLPE= *A. marmelos* leaves petroleum ether extract; CHLAE= *C. hirsutus* leaves acetone extract; CHLAqCold E= *C. hirsutus* leaves aqueous cold extract; CHLAqHotE= *C. hirsutus* leaves aqueous hot extract; CHLEtAE= *C. hirsutus* leaves ethyl acetate extract; CHLME= *C. hirsutus* leaves methanol extract; CHLPE= *C. hirsutus* leaves petroleum ether extract; GALAE= *G. asitica* leaves acetone extract; GALAqCold E= *G. asitica* leaves aqueous cold extract; GALAqHotE= *G. asitica* leaves aqueous hot extract; GALEtAE= *G. asitica* leaves ethyl acetate extract; GALME= *G. asitica* leaves methanol extract; GALPE= *G. asitica* leaves petroleum ether extract; PLAE= *Populus* sp. leaves acetone extract; PLAqCold E= *Populus* sp. leaves aqueous cold extract; PLAqHotE= *Populus* sp. leaves aqueous hot extract. PLEtAE= Populus sp. leaves ethyl acetate extract; PLME= Populus sp. leaves methanol extract; PLPE= Populus sp. leaves petroleum ether extract

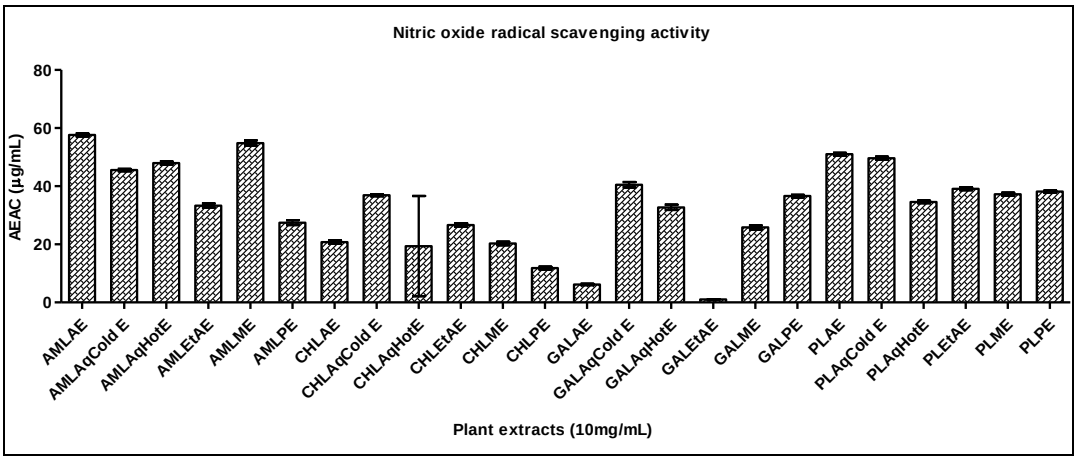

Fig 1: AEAC values of leaf extract from different plants as measured by Nitric oxide scavenging assay

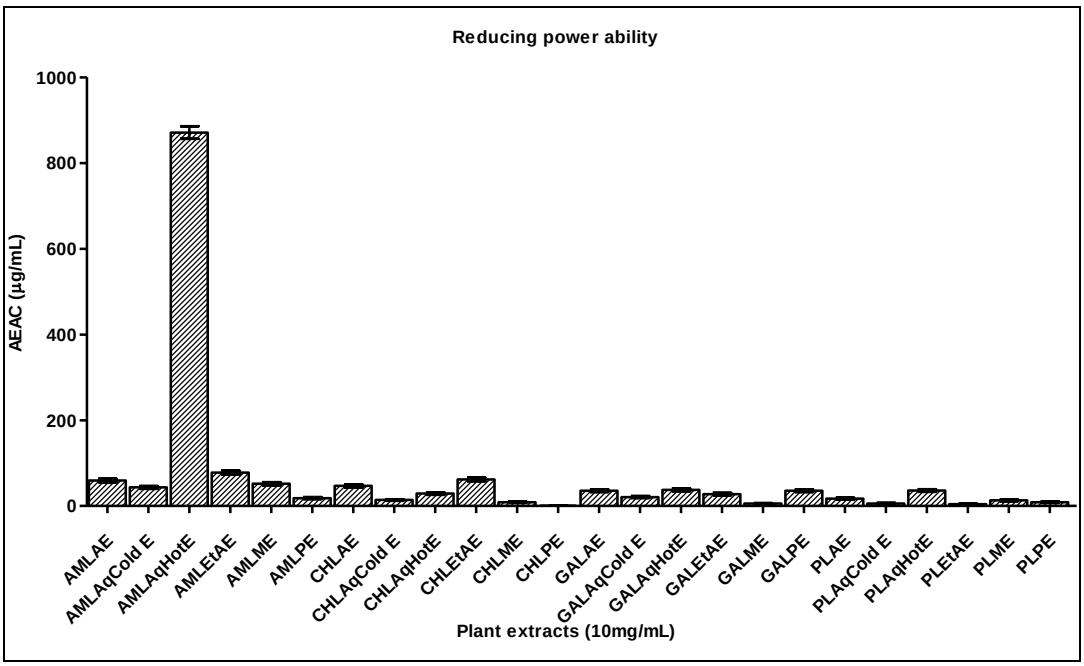

Fig 2: Reducing power ability of leaf extract from different plants represented as AEAC values.

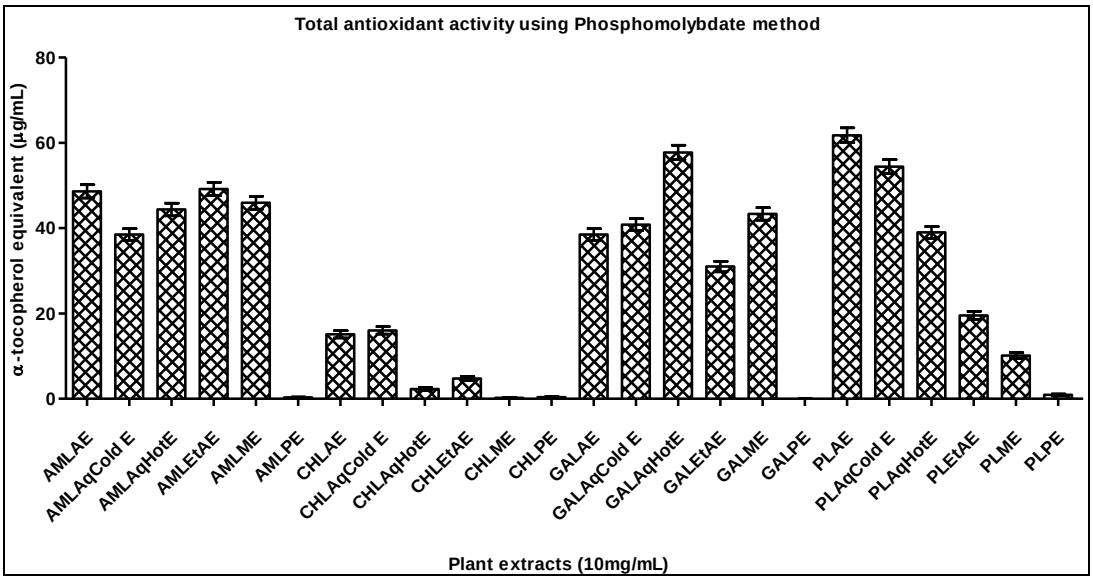

Fig 3: Total antioxidant potential of leaf extract from different plants represented as α tochopherol equivalent value, determined by Phosphomolybdate method.

Acknowledgement
The authors are thankful to JIBB, SHUATS**,** Allahabad and Boston College for Professional Studies, Gwalior for providing laboratory facilities and chemicals on timely basis for smooth running of the experiments. Authors extend their gratitude to Dr. Vijay Kumar, Head of Department, Department of Microbiology, RK. University, Rajkot, Gujarat, for helping in statistical analysis of experimental data.

References

1. Sabu MC, Kuttan R. Antidiabetic activity of *Aegle marmelos* and its relationship with its antioxidant properties. Indian Journal of physiology and pharmacology. 2004; 48(1):81-88.
2. Sudharameshwari K, Radhika J. Antibacterial Screening 0f *Aegle marmelos*, Lawsonia inermis And Albizzia libbeck. African journal of traditional, complementary and alternative medicines. 2007; 4(2):199-204.
3. Nayak S, Singhai AK. Antimicrobial activity of the roots of Cocculus hirsutus. Ancient science of life. 2003; 22(3):101.
4. Ganapaty S, Dash GK, Subburaju T, Suresh P. Diuretic, laxative and toxicity studies of Cocculus hirsutus aerial parts. Fitoterapia. 2002; 73(1):28-31.
5. Satyanarayana K, Mangathayaru V, Sreekanth J, Venkateswarlu V, Kokate CK. Studies on hypoglycemic and cardiotonic effects of roots of Cocculus hirsutus. Indian Journal of Pharmaceutical Sciences. 2001; 63(1):30.
6. Madhavan V, Ullah MS, Gurudeva MR, Yoganarasimhan SN. Pharmacognostical studies on the leaves of *Cocculus hirsutus* (Linn.) Diels–Chilahinta, an Ayurvedic drug. Indian Journal of Natural Products and Resources. 2010; 1(1):38-43.
7. Joshi P, Pandya P, Dei LP. Pharmacognostical and Phytochemical Evaluation of Grewia asiatica Linn (Tiliaceae) Fruit Pulp and Seed. International Journal of Pharmaceutical & Biological Archives. 2013; 4(2):333-336
8. Merghache D, Boucherit-otmania Z, Hacib I, Merghachec S, Chikhid I, Boucherita K. Antioxidant and antimicrobial activities of algerian *Populus nigra* L. buds extracts. Bioscience & engineering: an international journal. 2016; 3(1-2). DOI: 10.5121/BIOEJ.2016.3201.
9. Sreejayan N, Rao MNA. Nitric oxide scavenging by curcuminoids. Journal of pharmacy and Pharmacology. 1997; 49(1):105-107.
10. Yildirim A, Oktay M, Bilaloğlu V. The antioxidant activity of the leaves of Cydonia vulgaris. Turkish Journal of Medical Sciences. 2001; 31(1):23-27.
11. Fejes S, Blázovics A, Lugasi A, Lemberkovics É, Petri G, Kéry Á. *In vitro* antioxidant activity of Anthriscus cerefolium L. (Hoffm.) extracts. Journal of ethnopharmacology. 2000; 69(3):259-265
12. Jayaprakasha GK, Jena BS, Negi PS, Sakariah KK. Evaluation of antioxidant activities and antimutagenicity of turmeric oil: a byproduct from curcumin production. Zeitschrift für Naturforschung C. 2002; 57(9-10):828-835.
13. Reshma AK, Brindha P. *In vitro* anti-inflammatory, antioxidant and nephroprotective studies on leaves of *Aegle marmelos* and Ocimum sanctum. Asian J Pharm Clin Res, 2014; 7(4):121-129.
14. Panda BR, Mohanta SR, Manna AK, Si S. *In vitro* antioxidant activity on the aerial parts of Cocculus hirsutus Diels B. Journal of Advanced Pharmaceutical Research. 2011; 2(1):18-23.
15. Sinha J, Purwar S, Chuhan SK, Rai G. Nutritional and medicinal potential of Grewia subinaequalis DC.(syn. G. asiatica.) (Phalsa). Journal of Medicinal Plants Research. 2015; 9(19):594-612.
16. Kumar S, Bodla RB, Bansal H. Antioxidant Activity of Leaf Extract of *Aegle marmelos* Correa ex Roxb. Pharmacognosy Journal. 2016; 8(5):447-450.
17. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry*. 1999; 269(2):337-341.
18. Ramalingam R, Suganyadevi P, Aravinthan KM. Quantitative phytochemical analysis and their antioxidant activity of Cocculus hirsutus (l.) Diels fruit. International Journal of Phytomedicine. 2012; 4(4):447-455.
19. Brieger K, Schiavone S, Miller Jr FJ, Krause KH. Reactive oxygen species: from health to disease. Swiss medical weekly, 2012; 142:13659.
20. Partap S. A Review on Herbal Antioxidants. Journal of Pharmacognosy and Phytochemistry. 2012; 1(4):26-37.
21. Saxena M, Saxena J, Nema R, Singh D, Gupta A. Phytochemistry of medicinal plants. Journal of pharmacognosy and phytochemistry. 2013; 1(6):168-182.
22. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008; 4(2):89-96.
23. Goswami S, Jain R, Masih H. Antifungal, antioxidant and DNA protection potential of Grewia asiatica L. leaves acetone extract. Journal of Pharmacognosy and Phytochemistry, SP1. 2018, 212-217.
24. Picard S, Chenault J, Augustin S, Venot C. Isolation of a new phenolic compound from leaves of Populus deltoides. Journal of Natural Products. 1994; 57(6):808-810.
25. Duke JA. Amerindian medicinal plants. Typescript, 1983c.