



***In vitro* antioxidant studies of tuber of *Jatropha maheshwarii* Subram. & Nayar**

Gurusamy Sakthidevi¹, Veerabahu Ramasamy Mohan*¹, Salomon Jeeva²

¹Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu, India.

²Department of Botany, Scott Christian College (Autonomous), Nagercoil, Kanyakumari, Tamil Nadu, India.

*Corresponding author's E-mail: vrnohan@gmail.com

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ABSTRACT

Antioxidant activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of tuber of *Jatropha maheshwarii* have been tested using various antioxidant model systems viz., DPPH, hydroxyl, superoxide and ABTS. The ethanol extract of tuber of *Jatropha maheshwarii* showed potent DPPH, hydroxyl and Reducing abilities. The ethyl acetate extract exhibited potent ABTS radical cation scavenging activity. Methanol extract of the plant showed strong superoxide scavenging activity. The maximum inhibitory concentration (IC₅₀) in all models viz., DPPH, hydroxyl, superoxide and ABTS radical cation scavenging activity of tuber of *Jatropha maheshwarii* were found to be 18.98, 19.46, 19.23 and 19.02µg/mL respectively at 1µg/mL concentration. This study indicates significant free radical scavenging potential of the tuber of *Jatropha maheshwarii* which can be exploited for the treatment of various free radical mediated ailments.

Keywords: ABTS, DPPH, Flavonoid, *In vitro* antioxidant activity, *Jatropha maheshwarii*.

INTRODUCTION

Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions.¹ Free radicals have been implicated in the etiology of several major human ailments including cancer, cardio vascular diseases, neural disorders, diabetes, atherosclerosis, myocardial infarction and also in aging.²

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) have restricted use in foods as these synthetic antioxidants are suspected to be carcinogenic.³ Considering these ill effects, the need to search for natural antioxidants, especially of plant origin has greatly increased in recent years since plants are the most important source of natural antioxidants.⁴ Hence an extensive search for different types of antioxidants in various types of plants has been undertaken by various workers.

Many plant species have been investigated in the search for novel antioxidants^{5,6} but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and bioactive. Therefore, in recent years, considerable attention has been directed towards the identification of plants with antioxidant ability.

Jatropha maheshwarii Subram. & Nayar is an endemic plant commonly called as Athalai.⁷ It is distributed in the red clayey soils of southern east coast of Tamil Nadu, India and it is related to the petroplant, *Jatropha curcas* L. It is an erect shrub to 200cm with thick stem and underground root stock. The aerial part of the stem has a light green viscid fluid (latex) and it is used by the rural folk for curing various ailments like skin diseases, tooth

infections and haemorrhages. Perusal of the previous literature revealed that this medicinal plant is unexplored. However, no reports are available on the antioxidant activity of the tuber of *Jatropha maheshwarii*, therefore present investigation was undertaken to examine the total phenolic, flavonoid content and antioxidant activities of different solvent extracts of *Jatropha maheshwarii* tuber through various *in vitro* assay models.

MATERIALS AND METHODS

Collection of plant sample

Tuber of *Jatropha maheshwarii* Subram. & Nayar was collected from Vellamadam, Kanyakumari District, Tamil Nadu. With the help of local flora, voucher specimens were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu for further references.

Plant sample extraction

The coarse powder (100g) of *Jatropha maheshwarii* tuber was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 mL in a Soxhlet apparatus for 48 hrs. All the extracts were filtered through Whatman No.41 filter paper. All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for *in vitro* antioxidant activity. The methanol extract was used for the estimation of total phenolics and flavonoids.

Estimation of total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu method.⁸ Samples (100µL) were mixed thoroughly with 2 mL of 2% Na₂CO₃. After 2 min 100 µL of Folin-Ciocalteu reagent was added to the mixture. The



resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 743 nm against a blank. Total phenolic content was expressed as gram of gallic acid equivalents per 100 gram of dry weight ($\text{g } 100\text{g}^{-1} \text{ DW}$) of the plant samples.

Estimation of Flavonoid

The flavonoid content was determined.⁹ An aliquot of 0.5mL of sample (1mg/mL) was mixed with 0.1mL of 10% aluminium chloride and 0.1mL of potassium acetate (1M). In this mixture, 4.3mL of 80% methanol was added to make 5mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H.¹⁰

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method.¹⁰ Briefly, an 0.1mM solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 mL of the solution of all extracts in methanol at different concentration (50, 100, 200, 400 & 800 $\mu\text{g/mL}$). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0\} * 100$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell.¹¹ Stock solutions of EDTA (1mM), FeCl_3 (10mM), Ascorbic Acid (1mM), H_2O_2 (10mM) and Deoxyribose (10 mM) were prepared in distilled deionized water.

The assay was performed by adding 0.1mL EDTA, 0.01mL of FeCl_3 , 0.1mL H_2O_2 , 0.36mL of deoxyribose, 1.0mL of the extract of different concentration (50, 100, 200, 400 & 800 $\mu\text{g/mL}$) dissolved in distilled water, 0.33mL of phosphate buffer (50mM, pH 7.9), 0.1mL of ascorbic acid in sequence. The mixture was then incubated at 37°C for

1 hour. 1.0mL portion of the incubated mixture was mixed with 1.0mL of 10%TCA and 1.0mL of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Srinivasan *et al.*¹² The superoxide anion radicals were generated in 3.0 mL of Tris – HCL buffer (16 mM, pH 8.0), containing 0.5 mL of NBT (0.3mM), 0.5 mL NADH (0.936mM) solution, 1.0 mL extract of different concentration (50,100,200,400 & 800 $\mu\text{g/mL}$), and 0.5 mL Tris – HCl buffer (16mM, pH 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12mM) to the mixture, incubated at 25°C for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Antioxidant activity by radical cation (ABTS +)

ABTS assay was based on the slightly modified method of Huang *et al.*¹³ ABTS radical cation (ABTS+) was produced by reacting 7mM ABTS solution with 2.45 mM potassium per sulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS + Solution were diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of sample or trolox standard to 3.9 mL of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC). The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Reducing power

The reducing power of the extract was determined by the method of Kumar and Hemalatha.¹⁴ 1.0 mL of solution containing 50,100,200,400 & 800 $\mu\text{g/mL}$ of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH6.6) and potassium ferricyanide (5.0 mL, 1.0%). The mixture was incubated at 50°C for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5°C) in a refrigerator centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

Statistical analysis

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release



17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

RESULTS AND DISCUSSION

Total phenolic content and total flavonoid content

Phytochemicals have been of huge interest as a supply of natural antioxidants used for health promotion, food prevention, food flavoring and cosmetics as they are safer synthetics.¹⁵ The antioxidant activities of different extracts of *J. maheshwarii* tuber are in accordance with their amount of phenolic contents.

Phenols are very important plant constituents because of their scavenging ability, which is due to their hydroxyl groups. The phenolic compounds may contribute directly to the antioxidative action.¹⁶ The result indicates strong association between antioxidative activities and phenolic compounds suggesting that phenolic compounds are probably responsible for the antioxidative activities of *J. maheshwarii*. Phenolic compounds are effective hydrogen donors, making them good antioxidants. Thus, therapeutic properties of *J. maheshwarii* may be possibly attributed to the phenolic compounds present.

Table 1: IC₅₀ values of different solvent extracts of *Jatropha maheshwarii* tuber

Different solvent extracts	DPPH assay	Hydroxyl assay	Superoxide dismutase activity	ABTS assay
Petroleum ether	23.13	23.95	19.33	20.67
Benzene	20.11	23.04	23.97	19.08
Ethyl acetate	24.12	19.95	23.03	19.02
Methanol	19.67	20.12	18.45	21.23
Ethanol	18.98	19.46	22.18	22.48
Standard (Ascorbic acid)	20.05	24.94	22.76	-
Standard (Trolox)	-	-	-	19.29

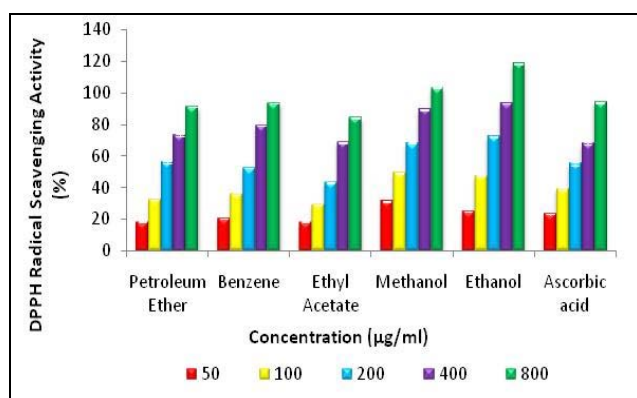


Figure 1: DPPH radical scavenging activity of different solvent extracts of tuber of *Jatropha maheshwarii*

Flavonoids are important secondary metabolites of plant modulating lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis. It has been confirmed

that pharmacological effects of flavonoids is correlating with their antioxidant activities.¹⁷ Flavonoids play vital role in scavenging the free radicals to prevent or reduce the oxidative damage encountered in human degenerative diseases.¹⁸

The total phenolic and total flavonoid content of the methanol extract of tuber of *Jatropha maheshwarii* were found to be 0.12g 100g⁻¹ and 0.99g 100g⁻¹.

DPPH radical scavenging activity

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *J. maheshwarii* tuber was shown in Figure 1. The scavenging effect increases with the concentration of standard and samples. Among the solvent tested, ethanol extract (800 µg/mL) exhibited highest DPPH radical scavenging activity (118.36%) with lowest IC₅₀ value (18.98 µg/mL). A higher DPPH radical scavenging activity is associated with a lower IC₅₀ value.

DPPH is a relatively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical, DPPH. From the present results it may be postulated that *J. maheshwarii* tuber reduces the radical to the corresponding hydrazine when it react with the hydrogen donors in the antioxidant principles.¹⁹ DPPH radical react with suitable reducing agents, the electrons become paired off and the solution loss colour stoichiometrically depending on the number of electrons taken up.²⁰

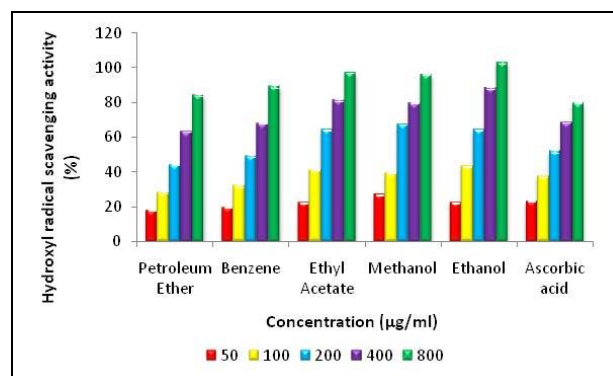


Figure 2: Hydroxyl radical scavenging activity of different solvent extracts of tuber of *Jatropha maheshwarii*

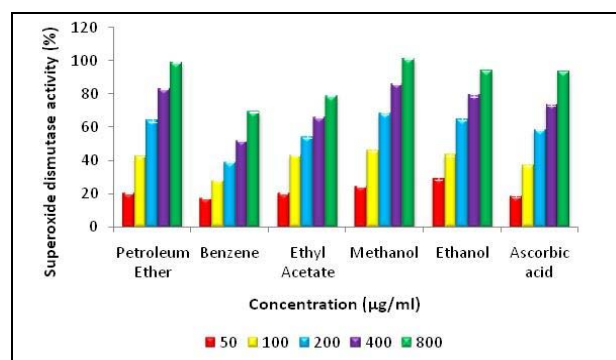


Figure 3: Superoxide radical scavenging activity of different solvent extracts of tuber of *Jatropha maheshwarii*

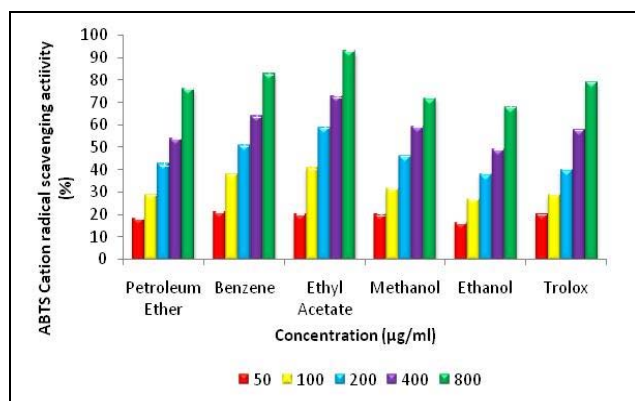


Figure 4: ABTS radical cation scavenging activity of different solvent extracts of tuber of *Jatropha maheshwarii*

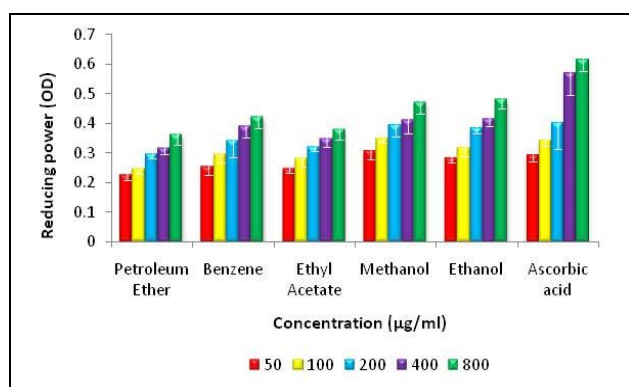


Figure 5: Reducing power ability of different solvent extracts of tuber of *Jatropha maheshwarii*

Hydroxyl radical scavenging activity of DPPH

Hydroxyl radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extract of *J. maheshwarii* tuber was presented in figure 2. Hydroxyl radical scavenging activity of different extracts of *J. maheshwarii* tuber increases with the concentration of extracts. At 800 µg/mL ethanol extract which has lowest IC_{50} value (19.46 µg/mL) showed highest scavenging activity (103.16%) against hydroxyl radical.

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage. They were produced in this study by incubating ferric-EDTA with ascorbic acid and H_2O_2 at pH 7.4, and reacted with 2-deoxy-2-ribose to generate a malondialdehyde (MDA)-like product. This compound forms a pink chromogen upon heating with TBA at low pH.²¹ When extracts of *J. maheshwarii* tuber were added to the reaction mixture, it removed the hydroxyl radicals from the sugar and prevented the reaction. Results showed the percentage of activity in a dose dependent manner. The ability of the above mentioned extracts to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and seems to good scavenger of active oxygen species, thus reducing the rate of the chain reaction.

Superoxide radical scavenging activity

The *J. maheshwarii* tuber extracts were subjected to the superoxide scavenging activity and the results were shown in figure 3. It indicates that methanol extract of *J. maheshwarii* tuber (800µg/mL) exhibited the maximum superoxide scavenging activity (94.13%) which is higher than the standard ascorbic acid whose scavenging effect (93.51%). IC_{50} values (Table 1) of petroleum ether, benzene, ethyl acetate, methanol and ethanol extract of *J. maheshwarii* tuber and standard in this assay were 19.33, 23.97, 23.03, 18.45, 22.18 and 22.76 µg/mL respectively.

Superoxide anion is also very harmful to cellular components.²² Robak and Glyglewski²³ reported that flavonoids are effective antioxidants mainly because they scavenge superoxide anions. As shown in figure 3, the superoxide radical scavenging activities of the plant extract and the reference compound are increased markedly with increasing concentrations. The results suggest that the plant extract is a more potent scavenger of superoxide radical than the standard ascorbic acid.

ABTS radical cation scavenging activity

The *J. maheshwarii* tuber extracts were subjected to ABTS radical cation scavenging activity and the results were presented in figure 4. The ethyl acetate extract exhibited potent ABTS radical cation scavenging activity (93.16%) in concentration dependent manner and ethanol extract has least scavenging activity (68.19%). A higher ABTS radical cation scavenging activity is associated with a lower IC_{50} value (19.02µg/mL).

The ABTS assay is based on the inhibition of the absorbance of the radical cation $ABTS^+$, which has a characteristic long wave length absorption spectrum.²⁴ The results imply that the extracts of *J. maheshwarii* tuber inhibit or scavenge the $ABTS^+$ radicals since both inhibition and scavenging properties of antioxidant towards $ABTS^+$ radicals have been reported earlier.

Reducing power

Figure 5 showed the reducing ability of different solvent extracts of *J. maheshwarii* tuber compared to ascorbic acid. The results clearly indicate that the reducing power of the *J. maheshwarii* tuber extracts increased in dose dependent manner. Among the solvent tested, ethanol extract exhibited higher reducing activity (0.481OD).

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity.²⁵ Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants.²⁶ The reducing capacity of *J. maheshwarii* tuber is a significant indicator of its potential antioxidant activity.

CONCLUSION

Currently, there has been an increased interest worldwide to identify antioxidant compounds from plant sources which are pharmacologically potent and have small or no side effects for use in protective medicine and food industry. Increasing acquaintance in antioxidant phytoconstituents and including them in daily uses and diet can give sufficient support to human body to fight those diseases. This study affirms the *in vitro* antioxidant potential of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of tuber of *J. maheshwarii*, with results comparable to those of the standard compounds such as ascorbic acid and trolox.

Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the tuber extract. In addition, the methanol extract of tuber was found to contain a noticeable amount of total phenolics and flavonoids, which play a major role in controlling antioxidants.

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REFERENCES

- Mishra A, Bapat MM, Tilak JC, Devasagayam TPA, Antioxidant activity of *Garcinia indica* (Kokam) and its syrup, *Curr. Sci.*, 91, 2006, 90-93.
- Badami S, Gupta MK, Suresh B, Antioxidant activity of the ethanolic extract of *Striga orobanchioides*, *J. Ethnopharmacol.*, 85, 2003, 227-230.
- Madavi DL, Salunkhe DK, Toxicological aspects of food antioxidants. Food antioxidants, New York. Marcl Dekker Inc, 1995, 267.
- Tsimogiannis D, Stavrakari M, Onepoulac V, Isolation and characterization of antioxidant components from Oregano (*Origanum heracleotium*), *Int. J. Food Sci Technol.*, 41, 2006, 39-48.
- Chu YH, Chang CL, Hsu HF, Flavonoid content of several vegetables and their antioxidant activity, *J. Sci. Food Agric.*, 80, 2000, 501-506.
- Koleva II, Van Beek TA, Linssen J, de Groot A, Evstatieva LN, Screening of plant extracts for antioxidant activity: A comparative study on three testing methods, *Phytochemical Analysis*, 13, 2002, 8-17.
- Uthayakumari F, Sumathy M, Pharmacognostical studies on the endemic medicinal plant – *Jatropha maheshwarii* Subr. & Nayar (Euphorbiaceae), *Int. J. PharmTech Res*, 3, 2011, 2169-2174.
- Lachman J, Hamouz K, Orsak M, Pivec V, Potato tubers as a significant source of antioxidant human nutrition, *Rostl Vyr*, 46, 2000, 231-236.
- Eom SH, Cheng WJ, Hyoung JP, Kim EH, Chung MI, Kim MJ, Yu C, Cho DH, Far infra red ray irradiation stimulates antioxidant activity in *Vitis flexuosa* Thunb. Berries, *Kor. J. Med. Crop Sci.*, 15, 2007, 319-323.
- Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P, Antioxidant activity *in vitro* of selenium-contained protein from the se-enriched *Bifodobacterium animalis*, *Anaerobe*, 16, 2010, 380-386.
- Halliwell B, Gutteridge JMC, Aruoma OI, The deoxyribose method: a simple test to be assay for determination of rate constants for reaction of hydroxyl radicals, *Ana Biochem.*, 165, 1987, 215-219.
- Srinivasan R, Chandrasekar MJN, Nanjan MJ, Suresh B, Antioxidant activity of *Caesalpinia digyna* root, *J. Ethnopharmacol*, 113, 2007, 284-291.
- Huang MH, Huang SS, Wang BS, Sheu MJ, Hou WC, Antioxidant and anti-inflammatory properties of *Cardiospermum halicacabum* and its reference compounds *ex vivo* and *in vivo*, *J. Ethnopharmacol.*, 133, 2011, 743-750.
- Kumar RS, Hemalatha S, *In vitro* antioxidant activity of alcoholic whole plant extract and subfractions of *Alangium lamarkii* Thwaites, *J. Chem. Pharm. Res.*, 3, 2011, 259-267.
- Khasawneh MA, Elwy HM, Fawzi NM, Hamza AA, Chevidenkandy A.R., Hassan A.H. Antioxidant activity, lipoxygenase inhibitory effect and polyphenolic compounds from *Calotropis procera* (Ait). *R. Br., Res. J. Phytochem.*, 5, 2011, 80-88.
- Ismail Shareef M, Gopinath SM, Jagan Mohan Reddy P, Dayananda KS, Mandal A, Purushotham KM, Antioxidant and Free Radical Scavenging Activities of *Zingiber officinale*, *Int. J. Innov. Res. Sci. Engi. Technol.*, 3, 2014, 10084-10090.
- Mbaebie BO, Edeoga HO, Afolayan AJ, Phytochemical analysis and antioxidants activities of aqueous leaf bark extract of *Schotia latifolia* Jacq, *Asian Pac. J. Trop. Biomed.*, 2, 2012, 118-124.
- Kumar PT, Kalita P, Barman TK, Chatterjee TK, Maity S, Quantification of Total Flavonoid Content and antioxidant activity in comparison to a reference flavonoid as *in vitro* quality evaluation parameter for assessing bioactivity of biomarkers in herbal extracts or formulations, *JPR: Bio Med Rx: an Int. J.*, 1, 2013, 757-766.
- Sreejeyan N, Rao MNA, Free radical scavenging activity of curcuminoids, *Drug Res.*, 40, 1996, 169.
- Rice-Evans C, Miller NJ, Factors influencing the antioxidant activity determined by the ABTS radical cation assay, *Free Rad. Res.*, 26, 1997, 195.
- Sharma A, Bhot M, Chandra N, *In vitro* antibacterial and antioxidant activity of *Bryophyllum pinnatum* (Lam.) Kurz, *Int. J. Pharm. Pharmaceu. Sci.*, 6, 2014, 558-560.
- Korycka-Dahl M, Richardson T, Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and amino acids, *J. Dairy Sci.*, 61, 1978, 400-407.
- Robak J, Gryglewski IR, Flavonoids are scavengers of superoxide anions, *Biochem. Pharmacol.*, 37, 1988, 837-841.
- Baskar R, Rajeswari V, Sathgishkumar T, *In vitro* antioxidant studies in leaves of *Annona* species, *Indian J. Experimen. Biol.*, 45, 2007, 480-485.
- Oktay M, Gulcin I, Kufrevioglu OI, Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts, *Leb-Wissen Technol.*, 36, 2003, 263-271.
- Yen GC, Chen HY, Antioxidant activity of various tea extracts in relation to their antimutagenicity, *J. Agric. Food Chem.*, 43, 1995, 27-32.

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