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Comparative antioxidant activity study of aqueous extract of *Tabernaemontana divaricata* leaves

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Abstract
Antioxidants are the compound that can prevent or inhibit oxidation which is a chemical reaction can produce free radical. *Tabernaemontana divaricata* plant has the ability to cure a variety of diseases with antioxidant capacity because of the presence of phytochemicals in it. The antioxidant activities of *T. divaricata* leaf aqueous extracts with different concentrations were investigate using DPPH assay, free radical scavenging activity, nitric oxide assay and superoxide radical scavenging activity. DPPH assay and nitric oxide assay shows higher values than free radical scavenging activity and super oxide antioxidant activity values for antioxidant capacity aqueous leaf extract of *T. divericata*. This novel study evident that aqueous extract of *T. divaricata* leaves may be exploited as an important source of antioxidants.

Keywords: *Tabernaemontana divaricata*, antioxidant activity**,** DPPH assay, free radical scavenging activity, nitric oxide assay, Superoxide radical scavenging activity

1. Introduction
Tabernaemontana divaricata belongs to the Apocynaceae family, growing evidence suggests that this plant has medicinal benefits and is used to treat various diseases such as diarrhoea, urinary disorders, abdominal tumours, epilepsy, eye infections, inflammation, leprosy, paralysis, rabies, rheumatic pain, skin diseases, ulceration and vomiting [1]. The leaf extracts could possibly be used as pharmacological interventions in many different ailments because of the antioxidants and potential secondary metabolites present in the leaves [2]. Antioxidants are the compound that can prevent or inhibit oxidation which is a chemical reaction can produce free radical. These free radical leads to chain reaction that damages the cells of the organism which also can initiate certain cancer [3].
Plants produce a miscellaneous group of secondary metabolites with antioxidant capacity. Antioxidants block the action of free radicals which have been concerned in the pathogenesis of numerous diseases [4-5]. Due to existence of phenolic compounds and flavonoids, plant holds antioxidant activity on human fitness. Phenols, flavonoids and tannins are act as antioxidant compounds which play a role as free radical scavengers [6].
The antioxidant such as thiols or ascorbic acid can diverse the chain reaction and prevent cancer. Certain level of antioxidant vitamin in diet is required for good health. The antioxidant agents also act as an anti-aging agent. Antioxidants are identified as molecules or compounds that regulate the process of auto oxidation either by intersecting the movement of free radicals or directly constraining their formation [7]. Medicinal plants are often recognized for their rich source of antioxidants, which include phenolic acids, phenolic diterpenes, flavonoids, volatile oils, carotenoids, and anthocyanidins [8]. These compounds target free radicals by quenching oxygen molecules, breaking antioxidant chains, donating hydrogen molecules, or acting as reducing agents [9-10]. Therefore, antioxidants are suggested to decrease oxidative stress, improve immune function, and increase healthy longevity. Several factor scan alter the antioxidant capacity of a certain species; these include the rate of reaction between the samples and the reactive species and the concentration ratio between the antioxidant and the target [11].
T. divaricata plant has the ability to cure a variety of diseases with antioxidant capacity because of the presence of phytochemicals in it [12]. In this study, The antioxidant activities of *T. divaricata* leaf aqueous extract with different concentrations were investigate using DPPH assay, free radical scavenging activity, Nitric oxide assay and Superoxide radical scavenging activity.

2. Methodology
Leaves of *T. divaricata* were collected from local area of Bhopal and collected leaves was authenticated by Botanist, Safia College of Science, Bhopal, Madhya Pradesh, India.

The drying of fresh leaves was carried out in sun but under the shade.

2.1. Extraction procedure
200 gm of shade dried leaves of *T. divaricata* were extracted in water by maceration method. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6×2 cm) and stored in a refrigerator (4 °C), till used for analysis [13-15].

2.2. *In-vitro* Antioxidant Activity of Extract leaves of T. divaricata
2.2.1. DPPH (1, 1-diphenyl-2-picrylhydrazyl) Radical Scavenging Assay
This assay measures by spectrophotometer the ability of antioxidants to reduce 2, 2-diphenylpicrylhydrazyl (DPPH), another radical not commonly found in biological systems. Stock solution (6 mg in 100 ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm [16]. The inhibition proportion was evaluated using the following formula:

Calculation of % Reduction = $(A_{control}-A_{test})/A_{control}\times100$

Where $A_{control}$ is the absorption (without extract) of the control and where A_{test} is the absorption in the presence of the extract / standard.

2.2.2. Free Radical Scavenging Activity (FRSA) using Hydrogen Peroxide
In Free Radical Scavenging Activity (FRSA) using Hydrogen Peroxide, 2 ml hydrogen peroxide (43 mol) and 1.0 ml aqueous leaf extract sample accompanied by 2.4 ml 0.1 M phosphate buffer (pH 7.4) Added. The resulting solution was maintained for 10 minutes and the absorbance at 230 nm was recorded. Without adding hydrogen peroxide, blank was ready and control was prepared without sample. It was used as a conventional compound with ascorbic acid [17]. The inhibition proportion was evaluated using the following formula:

Calculation of % Reduction = $(A_{control}-A_{test})/A_{control}\times100$

Where $A_{control}$ is the absorption (without extract) of the control and where A_{test} is the absorption in the presence of the extract / standard.

2.2.3. Nitric Oxide Scavenging Activity
Nitric oxide scavengers compete with oxygen resulting in decreased nitric oxide produced from sodium nitroprusside and Griess reagent was measured. Sodium nitroprusside spontaneously produces nitric oxide in aqueous solution at physiological pH, interacting with oxygen to generate nitric ions that can be estimated using Griess reagent [18]. Sodium nitroprusside (10 mmol/ L) was mixed with various extract concentrations in phosphate buffer saline (PBS) and incubated at 25 °C for 150 min. Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthyl ethylenediamine dihydrochloride) was added to the specimens. The chromophore absorbance created during the diazotization of sulphanilamide nitrite and subsequent coupling with napthyl ethyleneediamine was read at 546 nm and referred to the absorption of conventional ascorbic acid solutions treated in the same manner with Griess reagent as a positive control. The inhibition proportion was evaluated using the following formula:

Calculation of % Reduction = $(A_{control}-A_{test})/A_{control}\times100$

Where $A_{control}$ is the absorption (without extract) of the control and where A_{test} is the absorption in the presence of the extract / standard.

2.2.4. Superoxide radical scavenging assay
Superoxide radical (O_2) scavenging activity of extracts was measured by reported NBT reagent assay method. This assay is based on the generation of O_2 by auto oxidation of hydroxylamine hydrochloride in the presence of NBT, which gets reduced to nitrite [19-20]. Sodium carbonate (1 ml, 50 mM), NBT (0.4 ml, 24 mM), and EDTA (0.2 ml, 0.1 mM) solutions were added in the test samples of leaf extract or vitamin C (1 ml, 10-100 µg/ml) and nitrite ion in the presence of EDTA produced a color that was measured at λ_{max} 560 nm. The addition of hydroxylamine hydrochloride (0.4 ml, 1 mM) initiated the reaction, incubated at 25±2 °C for 15 min, then absorbance was measured at λ_{max} 560 nm. The inhibition proportion was evaluated using the following formula:

Calculation of % Reduction = $(A_{control}-A_{test})/A_{control}\times100$

Where $A_{control}$ is the absorption (without extract) of the control and where A_{test} is the absorption in the presence of the extract / standard.

3. Results & Discussions
The antioxidant activities of aqueous extracts of *T. divaricata* leaf with different concentrations were investigate using DPPH assay, free radical scavenging activity, nitric oxide assay and Superoxide radical scavenging activity. Percentage Inhibition of ascorbic acid with compare to aqueous extract of *T. divaricata* leaf using DPPH assay are depicted in table 1 and shown in figure 1.

Table 1: % Inhibition of ascorbic acid and aqueous extract of *T. divaricata* leaf using DPPH Assay

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Aqueous extract
1	10	30.42	5.83
2	20	59.11	10.91
3	40	67.48	19.28
4	60	75.25	28.42
5	80	77.58	31.47
6	100	79.63	33.24
IC_{50}		18.69	142.21

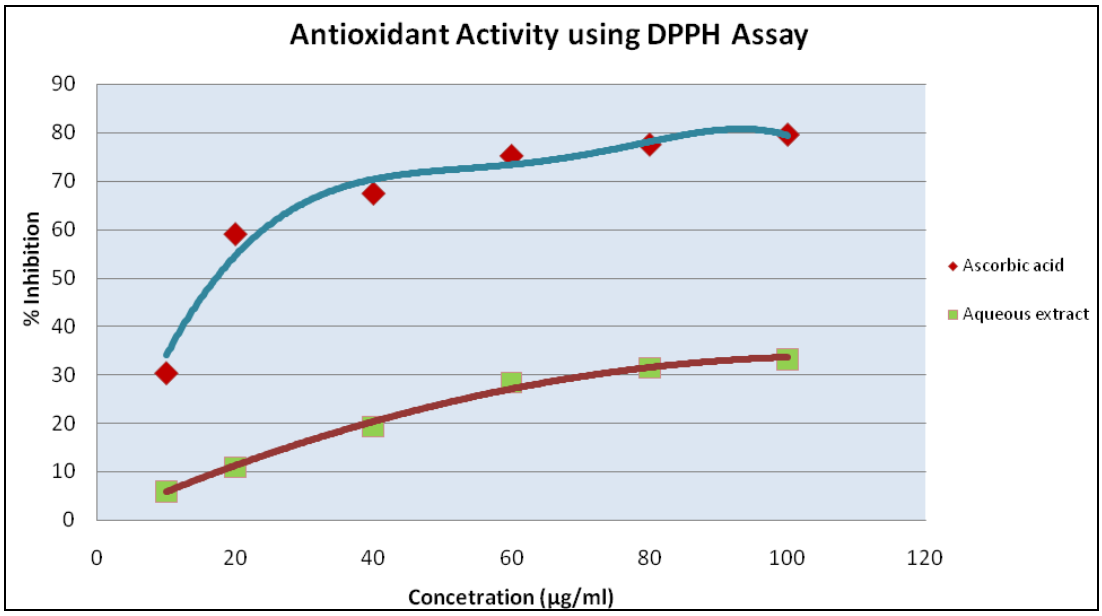

Fig 1: % Inhibition of ascorbic acid and aqueous extract of *T. divaricata* leaf using DPPH Assay

Percent Inhibition of ascorbic acid with compare to aqueous extract of *T. divaricata* leaf using Free radical scavenging activity (FRSA) using hydrogen peroxide are depicted in table 2 and shown in figure 2.

Table 2: % Inhibition of ascorbic acid and aqueous extract of *T. divaricata* leaf using FRSA assay

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Aqueous extract
1	20	42.87	20.24
2	40	52.19	35.87
3	60	60.41	41.52
4	80	65.78	50.62
5	100	68.75	57.64
IC 50		36.61	79.84

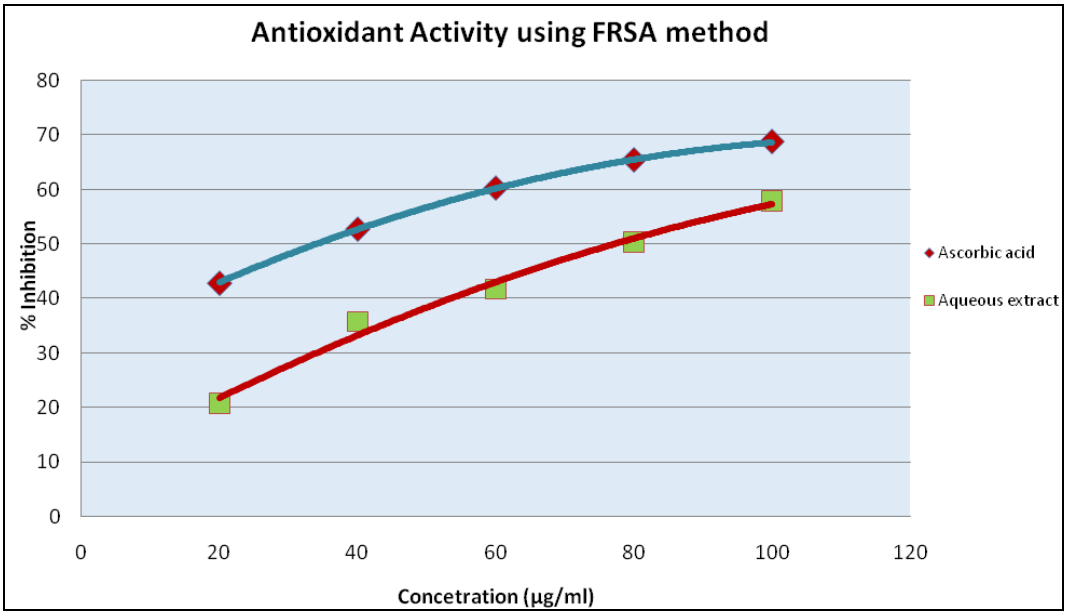

Fig 2: % Inhibition of ascorbic acid and aqueous extract of *T. divaricata* leaf using FRSA assay

Percent Inhibition of ascorbic acid with compare to aqueous extract of T. *divaricata* leaf using Nitric oxide scavenging activity are depicted in table 3 and shown in figure 3.

Table 3: % Inhibition of ascorbic acid and aqueous extract of *T. divaricata* leaf using nitric oxide assay

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Aqueous extract
1	25	30.52	10.25
2	50	47.7	17.74
3	75	52.92	19.35
4	100	67.43	20.96
5	125	68.89	24.51
6	150	87.42	33.87
IC 50		65.50	268.05

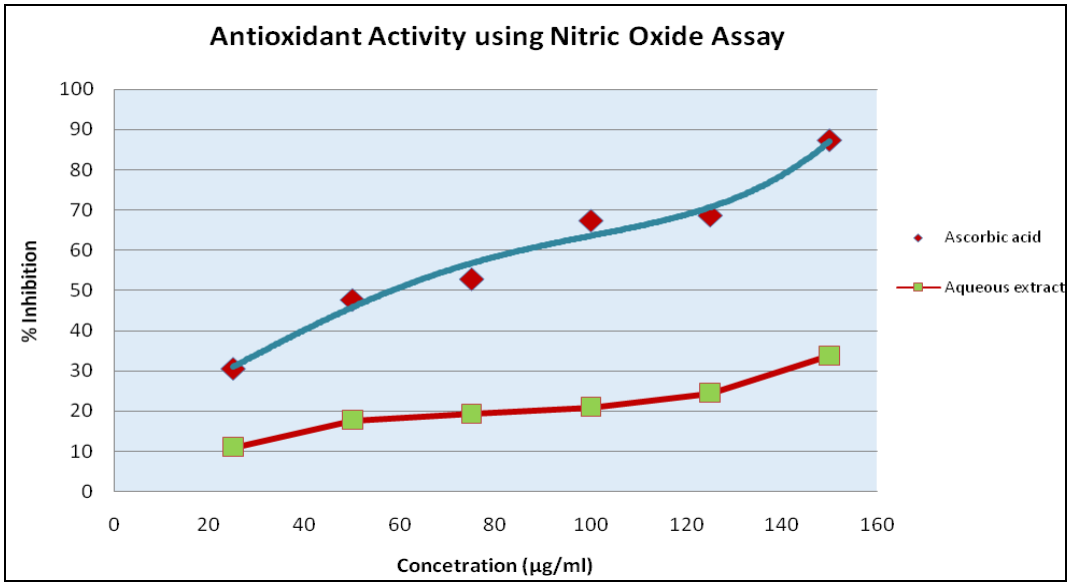

Fig 3: % Inhibition of ascorbic acid and aqueous extract of *T. divaricata* leaf using nitric oxide assay

Percent Inhibition of ascorbic acid with compare to aqueous extract of *T. divaricata* leaf using Superoxide radical scavenging activity using hydrogen peroxide are depicted in table 4 and shown in figure 4.

Table 4: % Inhibition of ascorbic acid and aqueous extract of *T. divaricata* leaf using superoxide radical scavenging activity

S. No.	Concentration (µg/ml)	Ascorbic Acid (% Inhibition)	Aqueous extract (Leaves) (% inhibition)
1	10	37.386	11.094
2	20	52.128	26.292
3	40	59.726	37.386
4	60	67.325	39.514
5	80	77.964	59.726
6	100	82.979	64.742
IC $_{50}$ Value		24.38	69.85

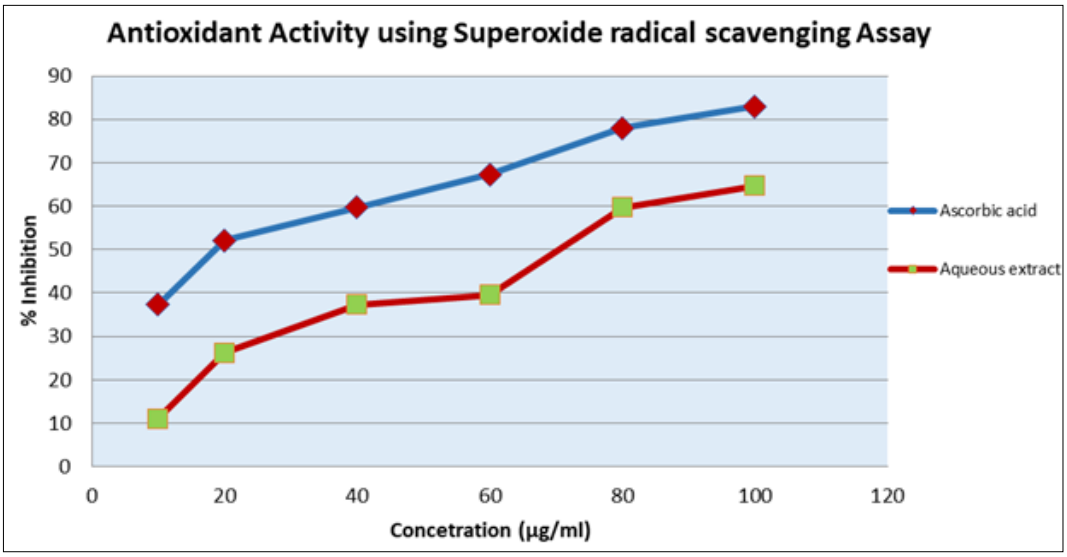

Fig 4: % Inhibition of ascorbic acid and aqueous extract of *T. divaricata* leaf using Superoxide radical scavenging activity

Comparative IC_{50} values of different assays for antioxidant activity study of aqueous extract of *T. divaricata* leaf are depicted in table 5.

Table 5: Comparative results of different methods for antioxidant activity study

S. N.	Antioxidant Activity assays	IC_{50} value of Ascorbic Acid	IC_{50} value of Aqueous Extract
1.	DPPH Assay	18.69	142.21
2.	Free radical scavenging activity	36.61	79.84
3.	Nitric Oxide Assay	65.50	268.05
4.	Superoxide Radical Scavenging Activity	24.38	69.85

Higher concentration resolute was unswervingly relative to greater antioxidant potential of the aqueous leaf extract. Among the assays tested for antioxidant capacity of aqueous leaf extract of *T. divericata*, DPPH assay and nitric oxide assay shows higher values than free radical scavenging activity and super oxide antioxidant activity values.

The existence of antioxidant, in the various extract have been shown to be an impart antioxidant activity by breaking the free radical chain [21]. This is because of the secondary metabolites like phenols, alkaloids and flavonoids present in the extracts due to which the antioxidants are the compounds capable of donating electron or hydrogen molecule for reduction [22].

4. Conclusion

This study evident that *T. divaricata* leaves may be exploited as an important source of antioxidants. Antioxidant capacity of aqueous leaf extract of *T. divericata*, DPPH assay and nitric Oxide assay shows higher values than Free radical scavenging activity and Super oxide antioxidant activity values. This novel study has gathered experimental evidence that aqueous extract of *T. divaricata* leaves are more potent as natural antioxidant.

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6. References

1. Bhadane BS, Patil MP, Maheshwari VL, Patil RH. Ethnopharmacology, phytochemistry and biotechnological advances of family Apocynaceae: A review. Phytother Res. 2018;32:1181-1210.
2. Flavonoids. Linus Pauling Institution, Oregon State University, Corvallis; 2016.
3. Lemmo W. Potential interactions of prescription and over-the-counter medications having antioxidant capabilities with radiation and chemotherapy. Intel J Can. 2014;137(11):2525-2533.
4. Aruoma OI. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutation Res. 2003;523(524):9-20.
5. Dasgupta N, De B. Antioxidant activity of *Piper betle* L. Leaf extract *in vitro*. Food Chem. 2004;88(2):219-224.
6. Coruh N, Celep AGS, Ozgokce F. Antioxidant properties of *Prangos ferulacea* (L) Lindl, *Chaerophyllum macropodum* Boiss and *Heracleum persicum* Desf. from Apiaceae family used as food in Eastern Anatolia and their inhibitory effects on glutathione-S-transferase. Food Chem. 2007;100(3):1237-1242.
7. Fierascu RC, Ortan A, Fierascu IC, Fierascu I. *In vitro* and *in vivo* evaluation of antioxidant properties of wild-growing plants. A short review. Curr Opin Food Sci. 2018;24:1-8.
8. Toghueo RMK, Boyom FF, Endophytes from ethno-pharmacological plants: Sources of novel antioxidants-A systematic review. Biocatal. Agric Biotechnol. 2019;22:101430.
9. Shori AB. Screening of antidiabetic and antioxidant activities of medicinal plants. J Integr Med. 2015;13(5):297-305.
10. Ahmed AA, El-Hag Ahmed MA, Abouhaddaf RM. Density functional investigation of the adsorption of nitric oxide on palladium clusters (PDN n=1-6). Int. J Adv. Chem. Res. 2020;2(1):05-08. DOI: 10.33545/26646781.2020.v2.i1a.15
11. Silveira D, De Melo AF, Magalhães PO, Fonseca-Bazzo YM. *Tabernaemontana* Species: Promising Sources of New Useful Drugs. Stu Nat Prod Chem. 2017;54:227-289.
12. Kalaimagal C, Umamaheswari G. Qualitative and Quantitative Analysis Using Aerial Parts from Double Flower Variety of *Tabernemontana Divaricata*. Int J of Ayur and Pharma Res. 2015, 31-36.
13. Khandelwal KR. Practical Pharmacognosy Technique and Experiments, 23rd ed. Nirali Prakashan, Delhi; c2005.
14. Kokate CK. Practical Pharmacognosy, 4th ed. Vallabh Prakashan, New Delhi, 1994.
15. Mukherjee PK. Quality Control of Herbal Drugs, 2nd ed. Business Horizons; 2007.
16. Olajuyigbe OO, Anthony JA. Phenolic Content and antioxidant property of the bark extract of *Ziziphus mucronata* wild. Subsp. *Mucronata* wild, BMC Comp Alter med. 2011;11(1):130.
17. Czochra MP, Widensk AJ. Spectrophotometric determination of H_2O_2 activity. Anal Chem Acta. 2002;452:177-84.
18. Marcocci L, Maguire JJ, Droy MT. The nitric oxide scavenging properties of *Gingo biloba* extract EGb 761. Biochem Biophys Res Commun. 1994;15:748-755.
19. Robak J, Gryglewski RJ. Flavonoids are scavenger of superoxide anions. Biochem Pharmacol. 1998;37(5):837-841.
20. Chanda S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. Afr J Micro Res. 2009;3(13):981-996.
21. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol. 1995;28(1):25-30.
22. Puranik SI, Hiremath MB, Nerli RB, Ghagane SC. Evaluation of *in vitro* Antioxidant and Anticancer Activity of *Tabernaemontana divaricata* Leaf Extracts against T-24 Human Bladder Cancer Cell Lines. Int J Cancer Res. 2018;14(2):100-108.
23. Magalhães LM, Segundo MA, Reis S, Lima JL. Methodological aspects about *in vitro* evaluation of antioxidant properties. Anal Chim Acta. 2008;613:1-19.