ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Phytochemical Properties and Antioxidant activity of Pollen of *Typha domingensis pers.* Grow in Marshes of Maysan Governorate Southern of Iraq

Majied Hassan Karbon[1], Raheem Jaafar Azeez[1], Zainab Yaseen Mohammed Hasan[2], Ali Nazar Yaseen[1*]

[1.] *Department of Science, College of Basic Education, Mustansiriyah University, Baghdad-Iraq.*

[2.] *Biotechnology Research Center/Al-Nahrain University, Baghdad-Iraq.*

***Corresponding Author: Ali Nazar Yaseen**

Abstract

The aim of this study was to evaluate the antioxidant activity of the *Typha domingensis Pers,* naturally growing pollen in the marshes of Maysan province in southern Iraq, and to link them effectively with the total flavonoids. (75 Ethanol+75 Water) for pollen these grains contain many effective plant compounds such as tannin, glucoside, alkali, saponin, polyphenols, and flavonoids. Flavonoids were extracted from pollen and detected using thin film chromatograph technique. The amount of flavons in the extract was estimated by chromatic method with aluminum chloride and was found to be approximately 71.3 milligrams per 50 grams of pollen powder. In determining antioxidant activity, the DPPH assay was used to show that the pollen extract had a good ability to inhibit DPPH free activity by 36.21% at 250 µg / ml of flavonoids compared to standard ascorbic acid (98.01% inhibition). The research concluded that the pollen extract contains the compounds of tannins, glycosides, alkalis, sapphones, flavonoids and phenolic compounds. Total flavonoids from pollen extract showed significant antioxidant activity.

Keywords: *Bardy, Typha domingensis Pers., Phytochemical, Phenolics, Flavonoids.*

Introduction

Typha domingensis pers. also named as Cattail is a member of the typhaceae family. These plants are herbaceous, perennial plants with long, slender green stalks ended with brown, fluffy, sausage like shaped flowering heads .The spike is bright yellow-to-orange-brown. The characteristic inflorescence gives the family the common name 'cat tails'.

This family is familiar in the warm temperature and tropical regions of the world, it's usually found in or near water. [1]. For this reason, Bardy (*Typha domingensis Pers*).Is very widespread in the marshes southern Iraq. Leaves of *Typha domingensis Pers.* possessed antimicrobial and haemostatic properties as evidenced by inhibit the growth of pathogenic bacteria and yeasts [2]. Another study showed that Typha domingensis, holds excellent potential to remediate heavy metals from municipal waste leachate and polluted water [3, 4]. In Turkish folk medicine the female inflorescences of *T.domingensis Pers* is used externally to treat wounds such as burns. Extracts of this fruit have been demonstrated to have wound healing properties in rat models [5].

Other than this, *Typha domingensis Pers pollen* is being eaten orally in Pakistan as antipyretic, increase flow of urine and to cure injuries [6]. The leaves of the plant have diuretic effect [7]. Pollen is also used in the treatment of nose bleeds, haematuria, uterine bleeding, dysmenorrhoea, gastralgia and postpartum abdominal pain [8]. In the marshes of southern Iraq, Khirret (pollen) is a pudding or sweet made from the pollen of this fruit [4].

Figure 1: The sweet (Khirret) from pollen of *Typha domingensis Pers.*

Peoples of Al-Ahwar (marshes) in the south of Iraq use pollen powder to increase the male fertility therefore called Viagra of Al-Ahwar [2]. The leaves extract of *Typha domingensis Pers.* grow in marshes south of Iraq had antimicrobial properties against and Gram-negative and Gram positive bacteria [2].Flavonoids and other phenolic compounds seem to be most valuable phytochemicals obtained from raw materials of herbs, seeds and fruits [9]. The aim of this study was to study the Phytochemical Properties and to evaluate antioxidant activity of pollen extract of Bardy (*Typha domingensis* Pers.) grow spontaneously in marshes of Masan governorate southern of Iraq, and to correlate it with total flavonoid compounds.

Materials and Methods

First: Pollen Material

The flour of *Typha domingensis pers.* Pollen was purchased from a local market in Masan governorate southern of Iraq.

Second: Extract the Total flavonoids from the Pollen *(Typha domingensis pers.*)

About (50) g of The flour of *Typha domingensis pers.* Pollen were placed in a 1 liter glass flask and then added 500 ml distilled water with (10% v/ v) HCl. Reflex extraction was performed for 8 hours continuously to ensure that the cleavage and broken of glycoside linkage of the flavonoids and the aglycon part was obtained. The pollen extract was filtered and cooled. The a glycan portion that is the biologic active part of flavonoids is extracted by ethyl acetate by adding 50 mL per each 50 mL extract and repeated three times using a separating funnel. Ethyl acetate is added to remove HCl residues used in extraction.

The Ethyl acetate layer is then dried using rotary evaporator at 45 ° C [10].

Third: Phytochemical Tests

For the purpose of these tests, 2.5 gm of the flour of *Typha domingensis pers.* Pollen macerated in 100 ml of 75% ethanol for overnight. Then filtered and the concentrated filtrate was identified for chemical components.

Detection of Tannins tests

To 0.5 ml of pollen extract solution 1 ml of water and 1-2 drops of 3% solution of the ferric chloride in distilled water was added. A greenish blue color was observed for Gallic tannins [11].

Detection of Glycosides

Mix 1 mL of the pollen extract with 2 ml of the Benedict reagent, place the mixture in a boiling bath for 5 minutes and left to cool. The red deposit indicated a presence of glycosides [12].

Detection of Alkaloids (Dragangroff Test)

Weigh about 60mg of Bismuth subnitrate and dissolved in 0.2 ml HCL, this is solution A. Solution B contains 600mg potassium iodide in 1 ml distill water. Mix the solution [A + B] and then add to the pollen extract, an orange to brown color will indicate the presence of alkaloids [13].

Detection of the Saponins

The detection process will be proceeding by shaking the pollen extract well. Formation of foam at the top will indicate presence of saponins [12].

Detection of Flavonoids

- (Alkaline reagent test) by using Sodium hydroxide solution is mixed with few amount pollenextract solution and left, a yellow color is obtained in presence of flavonoids.
- (Acidic test using a sulfuric acid): formation of orange-reddish color indicated the presence of flavonoids [14].

Detection of Polyphenolic Compounds

Using 1% ferric chloride solution to the pollen extract solution a brown deposition will formed [15].

Fifth: Determination of total Flavonoids

Qualitative Assay

A stock solution from the extracted total flavonoids was prepared by dissolving (50) mg residue in 10 ml of 50% ethanol to get a stock solution 5 mg/ml. A standard Rutin, Quercetin, Kaempferol and luteolin solutions were prepared in 50% ethanol also. Thin layer chromatography (TLC) was carried out using a silica coated plate (silica gel coated with flourescent indicator F254) with a thickness of 0.1 mm which represents the stationary phase in the chromatography separation process and the mobile phase contains: (n-Hexane: Ethyl acetate: Glacial acetic acid) (15:10:0.7). The TLC process is applied and the plate is placed in the mobile-saturated chamber as mentioned above. Flavonoids are left to be separated depending on their binding to the stationary-phase and solubility in the mobile phase.

When the mobile phase reached about three-quarters of the plate, plate was then removed and solvent front line was boarded. The type of flavonoids separated can be detected in corresponding to standard flavonoids spots in their distance that called RF value. This value is derived from dividing the distance travelled by each flavonoid in each model phase to the distance traveled by the solvent:

$$\text{Rf value} = \frac{\text{the distance traveled by each flavonoid}}{\text{distance traveled by the mobile phase}}$$

Each Flavonoid can be detected separately by the exposure of the silica plate to the UV light as a colored spot. The silica plate is covered with Fluorescent material, which flashes when it binds to the active groups of flavonoids under UV at a wavelength of 254 nm. The result is shown as bright spots under the UV light.

Quantitative Assay

Several Rutin standard solutions were prepared with concentration of (0.156, 0.3125, 0.625 and 1.25) mg/ml in 50% ethanol solution. The following interaction is performed:

About 1 ml of stock total flavonoids extract solution (5mg/ml) is transferred into test tubes, and 1 ml Rurin standard solutions of each concentration is placed in separated test tube. Add 1 ml of 5% sodium nitrite solution which dissolved in 50% ethanol to all tubes, then stirred and left at 25 ^{0}C for 5 minutes, then added to each test tube 2 ml of aluminum chloride at a concentration of 10% dissolved in 50% ethanol. Mix and leave for another 5 minutes at 25 ^{0}C. Finally, add 5 ml of 1N NaOH solution stir the mixture and then read the color with spectrophotometer at 510 nm [16]. A standard curve is then performed between reading absorbance of each standard solution with their known concentration to get the straight line equation and then calculating the concentration of the total amount of flavonoids in extracted pollen.

Sixth: Antioxidant Activity

Determination of DPPH frees Radical Scavenging

The free radical scavenging capacity of pollen extract of *Typha domingensis pers.* was determined using DPPH method [17] Freshly prepared DPPH (2, 2-diphenyl-1-picrylhydrazyl), solution was taken in glass test tubes and pollen extract was added followed by successive dilutions (50µg/ml to 250 µg/ml) to every test tube so that the final volume was 6 ml and after 0.5 hr., the absorbance was read at 517 nm using a uv-visible spectrophotometer. Control sample was prepared containing equal volume without any extract and standard .Ascorbic acid was used as standard and the absorbance was read at 517 nm using uv-visible spectrophotometer. Methanol was served as blank.

Results

First: Pollen Material

Second: Extract the Total flavonoids from the pollen *(Typha domingensis pers.*)

The resultant, semi dried, extract (0.665gm) was collected and saved to complete the rest of the analysis.

Third: Phytochemical Tests

Table 1: Phytochemical investigation of pollen of Typha *domingensis pers.* active compounds

Test	Result	Comments
Detection of Tannins	++	Greenish blue ppt.
Detection of glycosides	+++	Heavy Red ppt
Detection of alkaloids (Dragangroff)	+++	Little brown ppt
Detection of the saponines	++	Foam formation
Detection of Flavonoids	+++	Bright yellow
Detection of Polyphenolic compounds	+++	Brown ppt

Forth: Determination of Total Flavonoids

Qualitative Assay

Flavonoids were extracted from the pollen were identified by thin layer chromatography (TLC). Spots were obtained by UV exposure when exposing the plate to a 254 nm wavelength in comparison with standard flavonoids, Figure (2).

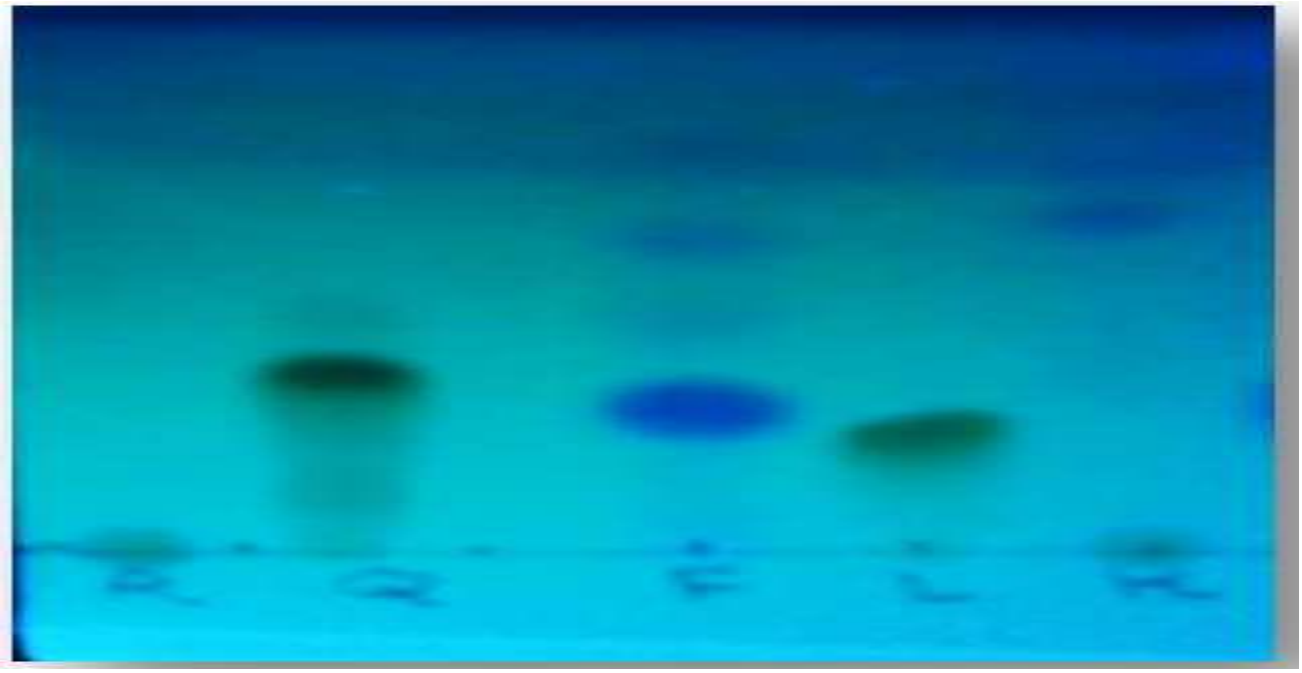

Figure 2: TLC chromatogram of the extracted total flavonoids (F), in corresponding to standard flavonoids; Rutin(R), Quercetin (Q), Luteolin(L), Kaempferol (K)

As shown in Fig (2) that the extract contains different flavonoids as quecetin, kaempferol, luteolin and others that can be detected in calculation of R_F values as in Table (2).

Table 2: The Values of Rf for different flavonoids and the extracted flavonoids

Flavonoid Type	Rutin	Quercetin	Luteolin	Kaempferol	Extracted flavonoids
R_F value	Base line	0.28	0.2	0.53	All spots & others

Quantitative Assay

The amount of flavonoids found in the pollen was estimated using the spectrometer at 510 nm depending on the concentration of the standard Rutin solution Table (3) where the straight line equation is obtained as shown in Fig(3).

Table 3: Absorption values of standard flavonoids Routine at different concentrations and total flavonoids of pollen extract

Absorption at (510 nm)	Rutin standard solution(mg/ml)
0.135	0.15625
0.341	0.3125
0.602	0.625
1.003	1.25
0.454	Total flavonoids of the pollen extract

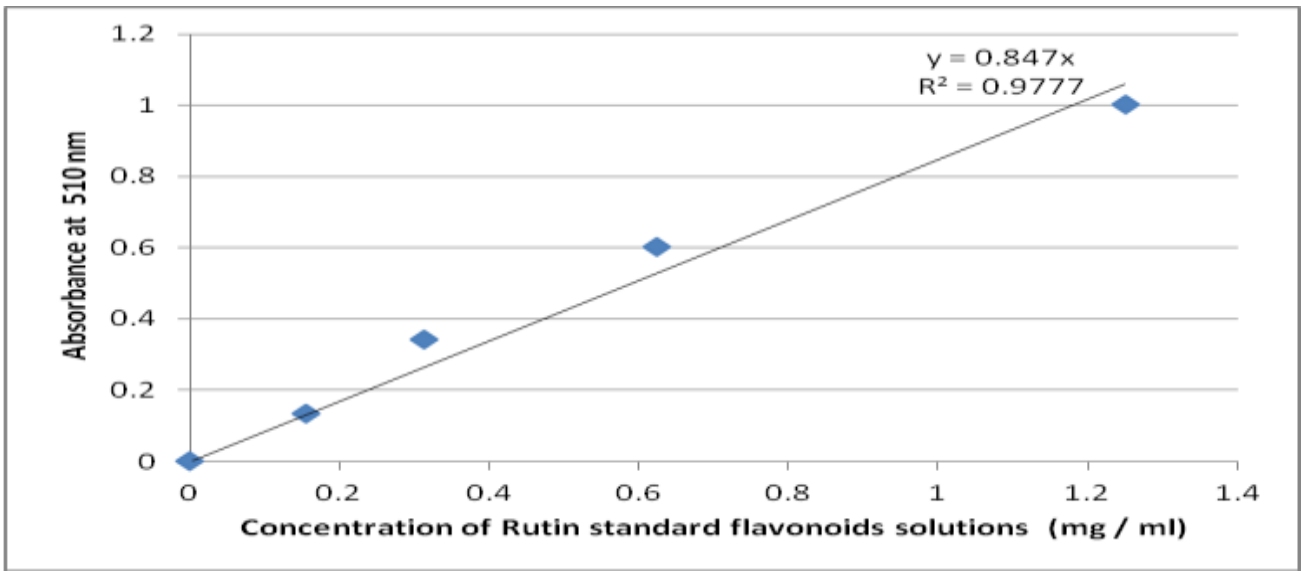

Figure 3: Rutin standard curve

From the equation of the straight line of the standard flavonoid curve to the Rutin with different concentrations, the concentration of the total flavonoids for the extract is as follows:

Y = 0.847 X

X = Y / 0.847

X = 0.454 / 0.847

X=0.536 mg/ml total flavonoids in each 5 mg residue

Residue weight was 0.665gm yielded from the extraction of 50 gm pollen

So: 0.536 X 665mg /5= 71.3 mg total flavonoid in each 50 gm of pollen.

Fifth: Antioxidant Activity

The ability of extract of pollen of *Typha domingensis pers.* to scavenge DPPH free radical was calculated as % inhibition which was found to be 36.21% at concentration 250 µg/ml, whereas % inhibition of ascorbic acid at the same concentration was 98.01% (Figure 4).

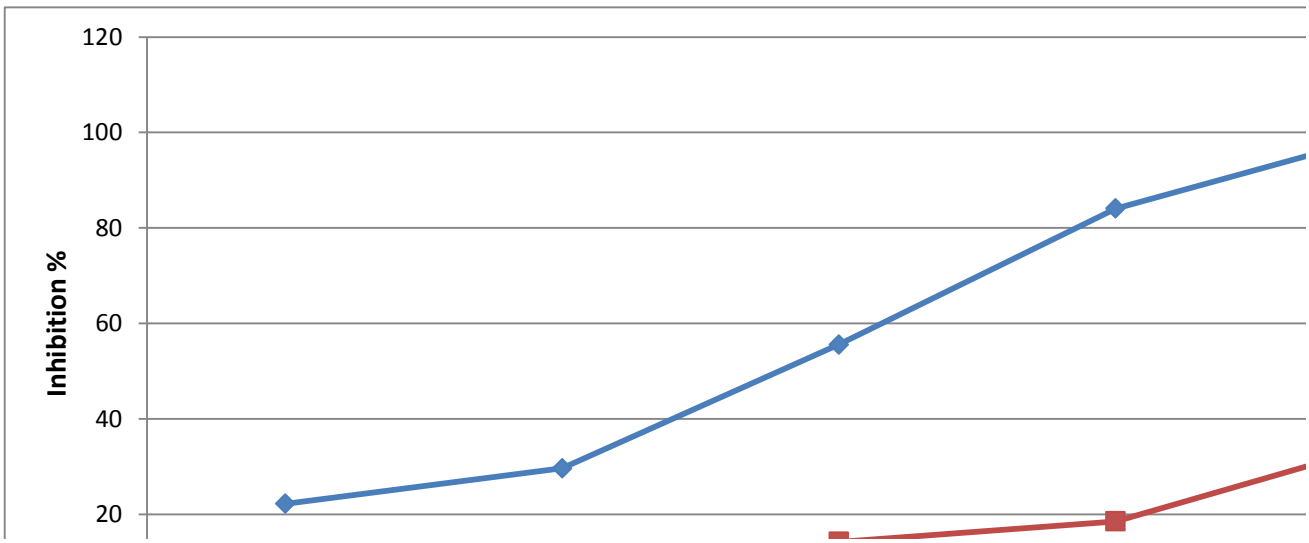

Figure 4: DPPH scavenging activity of extract of pollen of *Typha domingensis pers.*

Discussion

The phytochemical screening carried out on 75% ethanol extract of fruits of Typha domingensis pers. revealed the presence of phyto constituents such as, tannins, glycosides, alkaloids, saponins, flavonoids, and phenolic compounds. The correlation between saponins, flavanoids and phenolic compounds content and antioxidant capacity is a well-documented study [18].

Antioxidants fight free radicals and protect our body from various diseases by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. The use of DPPH free radical scavenging method provides rapid, easy and suitable method to evaluate the antioxidants activity [19].

DPPH is a purple color dye with absorption maxima of 517 nm and during reaction with a hydrogen donor (like pollen extract) the purple color disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance [20]. Pollen extract showed good electron donating capacity towards DDPH radical with percentage inhibition % 36.21, at concentration of 250 µg/ ml as compared to standard ascorbic acid (percentage inhibition 98.01) at same concentration. This in vitro assay indicate that pollen extract of *Typha domingensis pers* grows in marshes of Masan governorate southern of Iraq is a good source of natural antioxidant, which could be effective in preventing the progress of various diseases linked with oxidative stresses.

However, the components responsible for the antioxidants activity are not currently well-known. Therefore, further work need to be carried out to separate and identify the antioxidant compounds present in the pollen extract. Also, the in vivo antioxidant activities of the pollen extract needs to be assessed prior to clinical use.

Acknowledgments

The authors would like to thanks Mustansiriyah University and Al-Nahrain University Baghdad-Iraq for its support in the present works.

References

1. Long RW, Lakela O (1976).A Flora of Tropical Florida, Banyan Books, Miami Fl.
2. Al-Kalifawi EJ, Al-Azzawi YJ, Al-Fartousi KK, Musa HM (2017) "Physicochemical, phytochemical profiling and Biological activities of leaves extract of Bardy (*Typha domingensis Pers*.) from Al-Chibayish marshes in southern Iraq" Eighteenth International Conference - Istanbul University-Aydin 25-26 Istanbul-Turkey.
3. Mojiri A, Abdul Aziz H, Zahed MA, Aziz SQ, M Razip M, Selamat B (2013)" Phytoremediation of Heavy Metals from Urban Waste Leachate by Southern Cattail (*Typha domingensis*)" Inter. J. Sci. Res. Env. Sci., 1(4): 63-70.
4. Sri Lakshmi K, Hema Sailaja V, Anji Reddy M (2017) "Phytoremediation-A Promising Technique in Waste Water Treatment. Int. J. Sci. Res. and Manage, 5 (6): 5480-5489.
5. Akkol EK, Suntar I, Keles H, Yesilada E (2010) The potential role of female flowers inflorescence of *Typha domingensis Pers.* In wound Management. J. Ethno., 133(3):1027-1032.
6. Sardar AA, Khan Z, Perveen A, Farid S, Khan I (2014) In vitro antioxidant potential and free radical scavenging activity of various extracts of pollen of *Typha domigensis* Pers. Pak. J. Pharm. Sci., 27(2):279 284.
7. Duke JA, Ayensu ES (1985) Medicinal Plants of China Reference Publications, Inc., ISBN 0-917256-20-4.
8. Aliotta G, Della Greca M, Monaco P, Pinto G, Pollio A, Previtera, L (1990) In vitro algal growth inhibition by phytotoxins of *Typha latifolia.* J. Chem. Ecol., 16: 2637-2646
9. Abbasi MA, A Zafar, T Riaz, Aziz-ur Rehman, S Arshad, D Shahwar, M Jahangir, SZ Siddiqui, T Shahzadi, M Ajaib (2010) Evaluation of comparative antioxidant potential of aqueous and organic fractions of *Ipomoea carnea.* J. Med. Plant. Res., 4(18): 1883-1887.
10. Harborne JB (1984) Phytochemical methods, A guide to modern techniques of fruit analysis. Second edition, chapman and Hall, London, 169-172.

11. Iyengar MA (1995) Study of Crude Drugs. Manipal Power Press, Manipal, India, Edition 8th.

12. Siddiqui AA, Ali M (1997) Practical Pharmaceutical chemistry. CBS Publishers and Distributors, New Delhi, First Edition, 126-131.

13. Pouaklin A, Lacroix C, Roux N, Gangheyx GP, Deronin F (2000) Fongal contamination of food in Dermatology unit S.G. clean .Microbiology, 38: 4272-4273.

14. Oguyemi AO, Sofowora A (1979) Proceedings of a Conference on African Medicinal Plants, Ife-Ife. Univ. Ife, 20-22.

15. Edeoga HO, Okwu DE, Mbaebie BO (2005) Phytochemical constituents of some Nigerian medicinal Plant. African J. Biotechnology, 4(7): 685- 688.

16. Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64(4):555-559.

17. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I (2001) Antioxidant principles from *Bauhinia terapotensis.* J. Nat. Prod., 64: 892-895.

18. Audipudi AV, Bhaskar VS Chakicherla (2010) Anti-oxidative and Antimicrobial Activity of Methanol and Chloroform Extracts of *Gmelina Arborea* Roxb. International Journal of Biotechnology and Biochemistry, 6 (1): 139-144.

19. Argolo ACC, Santana AEG, Pletsch M, Coelho LCB (2004) Antioxidant activity of leaf extracts from *Bauhinia monandra.* Bio resource Technol., 95: 229- 233.

20. Ghoghari AM, Bagul MS, Anandjiwala S, Chauhan MG, Rajani M (2006) Free radical scavenging activity of *Aspidium cicutarium* rhizome. Journal of Natural Remedies, 6(2):131-34.

