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INFLUENCE OF PHENOLICS COMPOUND ON ANTHELMINTIC ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF *MACARANGA INDICA* WIGHT

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ABSTRACT

The present study aimed with the potential anthelmintic activity against *Tubifex tubifex* followed by *Taenia solium* with the ethanol leaves extract of *Macaranga indica* (Family: Euphorbiaceae) leaves at varied concentrations of 12.5, 25, 50 and 75 mg/ml. The study was compared with standard albendazole (50 mg/ml) as control. Initially, ethanol extract of plant leaves was subjected to phytochemical screening for the presence of various bioactive components followed by HPLC analysis for the presence of flavonoid compounds, quercetin. Anthelmintic activity study was carried out in terms of paralysis and death time on the above said test worms. The activity was compared with standard albendazole. Finally, the result revealed that dose dependent activity of the extract and the activity were may be due to the presence of quercetin in the extract.

Keywords: Albendazole, anthelmintic activity, *Macaranga indica*, *Taenia solium*, *Tubifex tubifex*

INTRODUCTION:

The demand for Medicinal and aromatic plants (MAPs) increases with the global acceptance by the people because of the inadequate availability, cost prohibitive disease treatments, unwanted toxic effects, and important development of resistance against infectious diseases by the Allopathic medicines. Among the various diseases and

infection, helminth infections are one of the most widespread human infections and its impact a significant section of the global population. The infection even though many times is neglected but in developing nations, they represent a serious risk to public health and increase the incidence of pneumonia, eosinophilia, anaemia, and malnutrition. They cause harm to the host by denying the host to access to food, resulting in blood loss, organ damage, intestinal or lymphatic obstruction, and secreting poisons. Helminthiasis is a leading cause of illness **[1, 2]**. Traditionally, there are many plants used for combating helminthiasis. Several plants with their active constituents showed effective anthelmintic activity. Off late, *Macaranga indica* (MI) (belongs to the family Euphorbiaceae) is such plant that widely available in road side in hilly areas especially in the Western Ghats regions in India. Apart from that, the plant is also distributed in tropics of Africa, other Asian countries, Australia, and the Pacific regions. Various bioactive components of the plant are like stilbenes, flavonoids, coumarins, alkaloids, terpenoids, and tannins that show versatile medicinal and therapeutic applications **[3]**. Scientific literatures revealed that the plant is used traditionally for the treatments of cuts, anaemia, paralysis, tumour, swellings, boils, bruises, and sores **[4, 5]**. Apart from that its additional therapeutic and pharmacological activities are well known in the market viz. anti-cancer, anti-inflammatory, anti-oxidant, anti-microbial, and anti-plasmodial activities **[6]** but the activities are limited. Due to unexplored information by the scientific data and less availability of raw material from the said plant, many research gaps are still there. Therefore, it is worthwhile to explore the plant in new direction of therapeutic application as anthelmintic activity.

MATERIALS AND METHOD:

Plant Material:

The fresh leaves of MI were collected in the month of August from Mangalore, Karnataka, India, and authenticated by Prof. P.E. Rajasekharan, Principal Scientist, Indian Institute of Horticultural research, Bangalore. The leaves are preserved as a voucher specimen (No. KCP-PCOG/MI-Leaves/2020-21/601) and submitted to the Pharmacognosy department for future reference.

Preparation of Extract:

The leaves of MI were shade dried for 21 days and observed for microbial contamination. Finally, the shade dried MI leaves (500 g) were subjected to ethanol extract after defatted with petroleum ether by soxhlet method at 45 degree C for 13 hrs.

The extract finally filtered and evaporated to dryness by water bath. The preliminary phytochemical investigation was carried out as per the standard method followed by TLC chromatography for separation and identification of the bioactive components present in the extract. Furthermore, HPLC study was performed to confirm the bioactive component with the estimated amount in the extract.

Phytochemical screening and chromatographic methods:

The presence of various group of bioactive constituents viz. alkaloids, glycosides, tannins, saponins, terpe noids, flavonoids, and sterols as per standard methods **[7]**. Thereafter, based on the group of constituents, further TLC chromatography was run for separation of individual compounds by using various solvents by compared with standard sample. For TLC, Toluene, ethyl acetate and formic acid (5:4:0.2) solvent system was used for detection of quercetin as per the standard method followed **[8]**. A stock solution of quercetin was prepared by taken 10 mg of quercetin (98.6 % purity) in ethanol and the volume filled with ethanol up to 1o ml. Sample solution was prepared by dissolved 100 mg of MI extract in 10 ml of ethanol (concentration of 10 mg/ml). It was then sonicated for 10 min.

Furthermore, the presence of most two bioactive constituents was confirmed with HPLC study and estimated the amount content in the extract. HPLC analysis was performed using Shimadzu liquid chromatogr -aphy systems.

MI plant extract was run after micro filtration with HPLC using a C18 column. The mobile phase was selected as methanol and 0.40% phosphoric acid (49:51, V/V), at 25℃. The flow rate was 1.5 ml/min, and the injection volume was 20 µl. The sample quercetin was detected at 265 nm at UV **[9]**. Sample and standard was prepared by dissolved 10 mg of sample and standard in the ethanol solvent and volume made up to 100 ml respectively. Sample further diluted 1 ml into 100 ml and run HPLC system.

Animal used:

Aquarium worm *Tubifex tubifex* was collected from fish aquarium shop, Bangalore local market and *Taenia solium* were procured from the pig slaughter house in local market, Bangalore **(Figure 1 and 2)**. MI plant extract was used in four different concentrations namely, 12.5, 25, 50 and 75 mg/ml for perform anthelmintic activity.

Figure 1: ***Taenia solium***

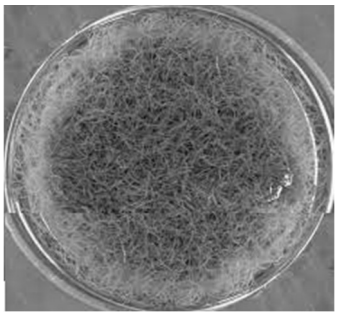


Figure 2: ***Tubifex tubifex***

Statistical analysis:

In the present study, all the data were reported as mean ± S.E.M. (n = 3). Data were analyzed using one way factorial ANOVA tests using SPSS followed by Dennett's tests on each group against control treatment. $P<0.05$ and $P<0.001$ were considered as statistically significant. Statistical program used was GRAPHPAD PRISM® (version 6.00; GraphPad Software Inc., San Diego, CA, USA) and Microsoft Excel, 2007, used for graphical presentation.

RESULT

Yield:

MI leaves were extracted with ethanol solvent and the yield was found to be 37.23 g (w/w). The per cent yield was found to be 7.45.

Preliminary phytochemical screening:

Various chemical tests were performed as per the standard method followed and revealed the presence of high content of alkaloids, steroids, flavonoids, poly phenolics, tannins, and saponins in leaves. Further, TLC was performed as per the method described above and reported the presence of quercetin in the extract **(Figure 3)**. Toluene, ethyl acetate and formic acid were used in combination and quercetin was separated with the Rf value 0.42.

Thereafter, HPLC study was performed for the extract and revealed the confirmed presence of Quercetin in the extract. Further, quantification of the constituent was carried out and reported the presence of 1.088 % in the extract **(Figure 4 and 5)**. Both the **Figures 4 and 5** showed the retention time at 2.844 min for standard quercetin and quercetin present in the extract both.

Anthelmintic study:

Four various concentration of the MI extract were prepared and initially performed with the aquarium worm *Tubifex tubifex* and observed the result for paralysis and complete death time required for the worm. It was revealed that higher concentration (75 mg/ml) of MI ethanol extract produced fast paralytic effect followed by the time to death for all worms **(Table 1)**.

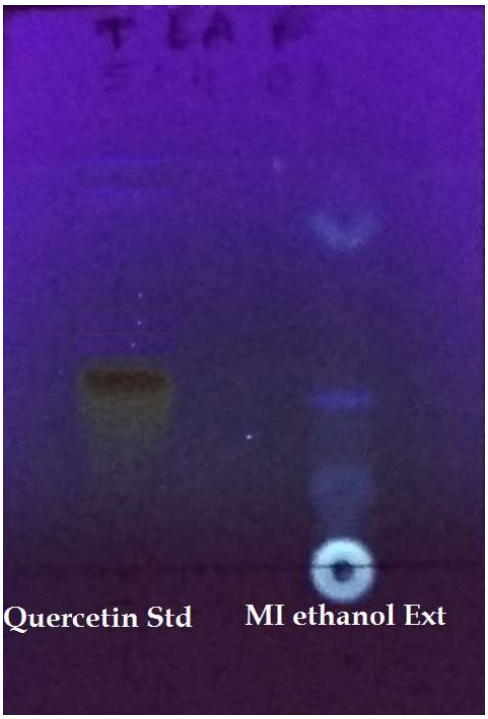


Figure 3: TLC of quercetin in MI extract

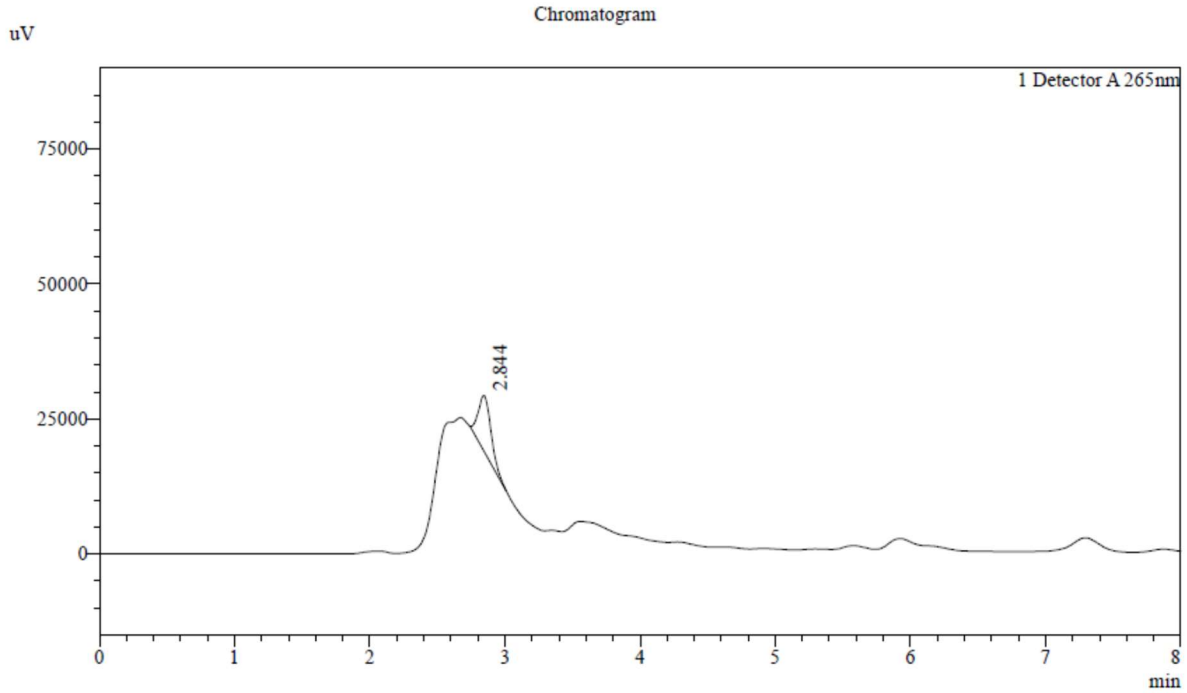


Figure 4: Presence of Quercetin in the MI extract by HPLC study

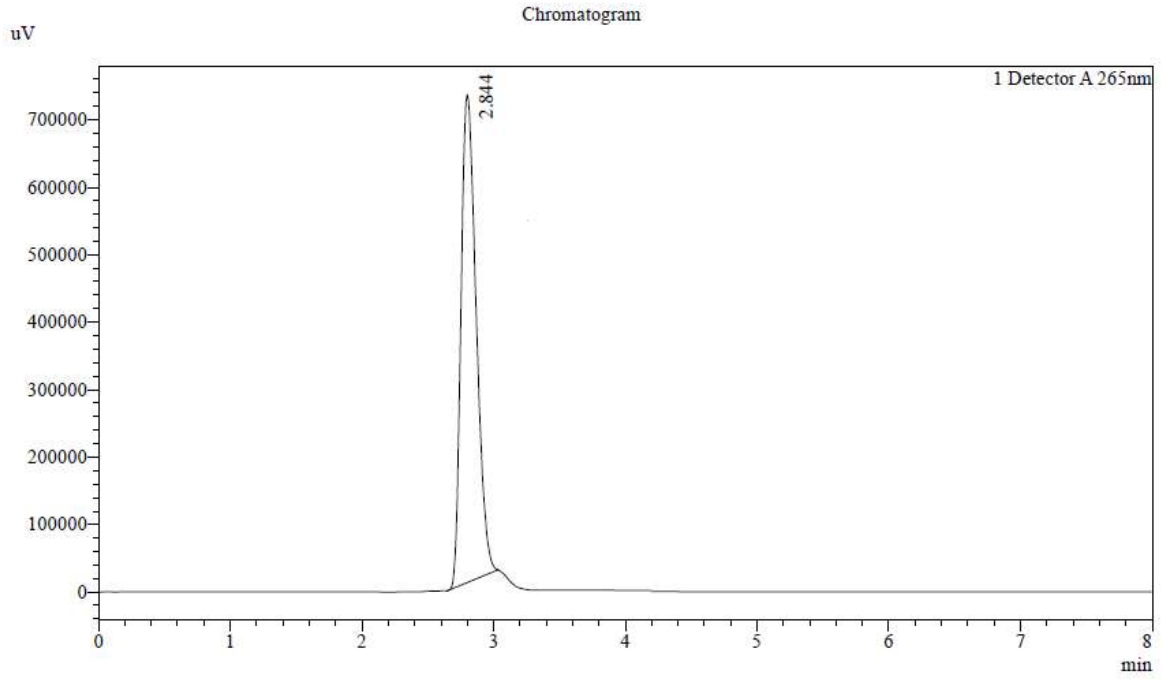


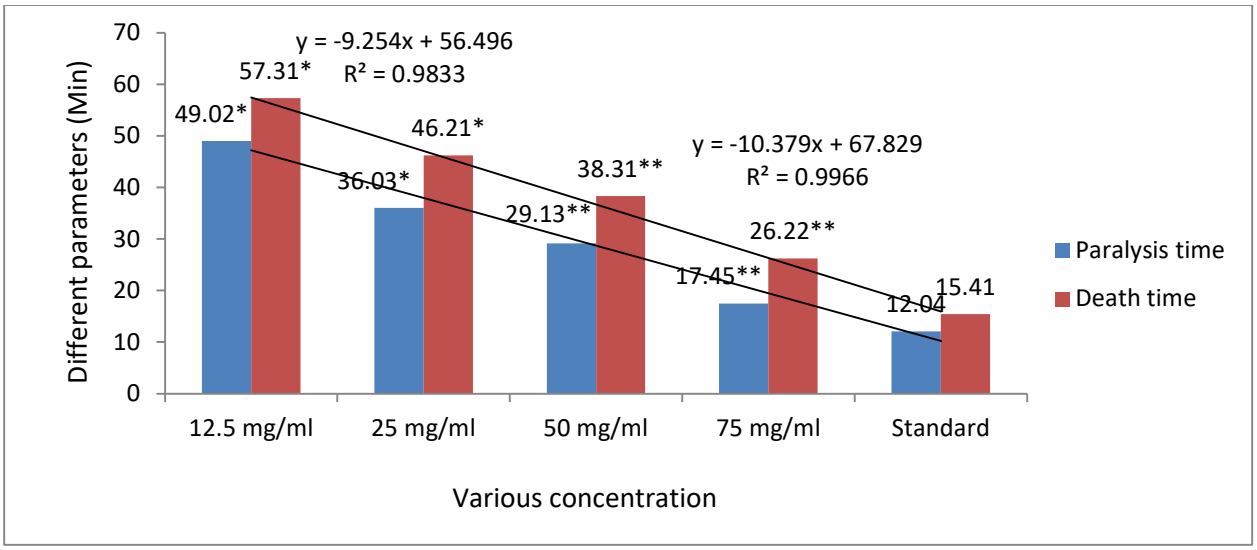
Figure 5: HPLC of Standard Quercetin

Table 1: Paralysis and death time of MI ethanol extract against *Tubifex tubifex*

Treatment Concentration (mg/ml)	Paralysis time (Min)	Death time (Min)
12.5	38.32 ± 0.32*	47.44 ± 0.19*
25	24.03± 0.12*	37.23 ± 0.42**
50	18.19 ± 0.36**	26.30 ± 0.01**
75	12.22 ± 0.05**	19.40 ± 0.53**
Standard 50 mg/ml	8.32 ± 0.07	18.44 ± 0.18

- **Values are mean ± SEM, (n = 3); *P < 0.05, **P <0.001, Dennett's test as compared to positive control (Albendazole)**

Further, the same activity performed with *Taenia solium* and also showed the similar result as given with *Tubifex tubifex*. The result was tabulated in **Figure 6**. The concentration dependent activity was observed where 75 mg/ml showed highest activity by reduced the paralysis time as well as death time than other concentrations. Death time (R^2= 0.996) showed statistically more significant than paralysis time (R^2 = 0.983).



- **Values are mean ± SEM, (n = 3); *P < 0.05, **P <0.001, Dennett's test as compared to standard (Albendazole)**

DISCUSSION

In the present study, ethanol solvent was used for the extraction of the leaves of MI plant. Many earlier scientific literatures reported that widely used solvent is ethanol because maximum amount of bioactive constituents are soluble in ethanol solvent and also content of yield is high **[10, 11]**. Even though, the solvent is relatively low toxic in nature. The same trend was followed for the present experiment.

A wide number of bioactive constituents are soluble in ethanol solvent and based on that various chemical tests were performed and revealed the presence of many group of constituents. Many earlier literatures revealed ethanol has the maximum ability to absorb glycosides **[12]**, polyacetylenes, sterols **[13]**, polyphenols, tannins, flavonols, terpenoids, and alkaloids **[14]** due to a protic organic nature with the polarity index value of 5.2 and a high dielectric constant of 24.55. Perhaps it also increased the yield as resulted in the present study **[15]**. In the present experiment alkaloids, tannins, flavonoids, sterols, polyphenolics, saponins were identified using the chemical tests of MI leaves which was similar to that of earlier study carried out for MI plant species **[16]**.

Thereafter, known flavonoid, quercetin was identified and separated by TLC method using the combination of solvent system which has greater impact on further therapeutic activity i.e. anthelmintic activity. Earlier literature showed that toluene, ethyl acetate and formic acid gave clear separation of quercetin from the plant extract **[17]**. The same type of separation resulted in the present study with the similar

type of retention factor. Furthermore, HPLC study was performed for quantification of quercetin in the extract using methanol and 0.40% phosphoric acid as mobile phase. The shorter retention time showed by the both standard quercetin and the quercetin present in the extract.

There are plenty chemicals available in markets for the treatment of internal worms but due to other side effects and toxicity causes the limitation in use of those synthetic medicines. Hence, people are inclined towards the herbal side for the root level curative diseases with very less side effects. By focused on that, in the present study, MI leaves were selected for the anthelmintic activity which was not reported by the earlier literatures. Apart from that, bioactive constituents from the extract of the MI leaves showed especially the therapeutic activity. Giving the concentration, particular flavonoid compound i.e quercetin was identified and correlated with the present study. It was reported that quercetin plays immense role in various therapeutic activity with its high antioxidant nature. Traditionally, MI leaves were reported for the treatment of wounds, stomach-ache, dysentery, etc. **[18]**. Based on the concept, the present anthelmintic activity was carried out for the first time. In this activity, quercetin also plays vital role by the generation of oxidative stress as well as alteration of the stress response enzyme activities. Not only that, quercetin also targets neuronal system of adult worms and causes a predominant change in metabolism **[19, 20]**. Hence, in the MI extract due to the presence of quercetin as one of the bioactive compound showed the potent anthelmintic activity.

CONCLUSION

The present study was performed to determine the potent anthelmintic activity of MI ethanol leaves extract against *Tubifex tubifex* followed by *Taenia solium* using various concentrations. Furthermore, the presence of Quercetin was identified and estimated using TLC and HPLC studies and extract was performed anthelmintic activity against standard albendazole. Quercetin was identified as one of the bioactive compound that acts by the mechanism by generated oxidative stress and alteration of the stress response enzyme activities. Finally, all the data supported in vitro efficiency of anthelmintic activity of MI ethanol extract of leaves.

REFERENCES

[1] Bundy DA. Immunoepidemiology of intestinal helmintic infection I: The global burden of intestinal nematode

disease. Trans R Soc Trop Med Hyg. 1994;8: 259–61.

[2] Das SS, Dey M, Ghosh AK. Determination of anthelmintic activity of the leaf and bark extract of *Tamarindus indica* linn. Indian J Pharm Sci. 2011;73(1):104-107.

[3] Magadula JJ. Phytochemistry and pharmacology of the genus Macaranga: A review. J Med. Plant Res. 2014; 8: 489-503.

[4] Uddin NS. Traditional uses of ethnomedicinal plants of the Chittagong hill tracts, Bangladesh. Dhaka; National Herbarium, Bangladesh: 2006: p-658.

[5] Jain A, Katewa SS, Choudhary BL, Galav P. Folk herbal medicines used in birth control and sexual diseases by tribals of southern Rajasthan, India. J Ethnopharmacol. 2004; 90: 171–7.

[6] Yang DS, Peng WB, Yang YP, Liu KC, Li XL, Xiao WL. Cytotoxic prenylated flavonoids from *Macaranga indica*. Fitoterapia. 2015; 103: 187-191.

[7] Das K, Khan SM, Sounder J, Mohan U, Prasad VS. Phytochemical screening and establishment of the antidiabetic potential of aqueous leaf extract of the endangered plant *Decalepis nervosa* in rats with alloxan-induced diabetes. Turk. J. Pharm. Sci. 2020; 17 (3): 319-328.

[8] Doshi GM, Une HD. Quantification of Quercetin and Rutin from *Benincasa hispida* Seeds and *Carissa Congesta* Roots by High-performance Thin Layer Chromatography and High-performance Liquid Chromatography. Pharmacognosy Res. 2016;;8(1): 37-42.

[9] Yue-ling M, Yu-jie C, Ding-rong W, Ping C, Ran X. HPLC Determination of Quercetin in Three Plant Drugs from Genus Sedum and Conjecture of the Best Harvest Time. Pharmacogn J. 2017; 9(6): 725-728.

[10] Abarca-Vargas R, Malacara CFP, Petricevich VL. Characterization of chemical compounds with antioxidant and cytotoxic activities in bougainvillea x buttiana holttum and standl, (Var. rose) extracts. Antioxidants. 2016; 5(45): 1–11.

[11] Hikmawanti NPE, Fatmawati S, Asri AW. The Effect of Ethanol Concentrations as The Extraction Solvent on Antioxidant Activity of Katuk (*Sauropus androgynus* (L.) Merr.) Leaves Extracts. Earth and Environmental Science. 2021; 755:

012060. doi:10.1088/1755-1315/755/1/012060.

[12] Houghton PJ, Raman A. Laboratory Handbook for the Fractionation of Natural Extracts. In Laboratory Handbook for the Fractionation of Natural Extracts. London: Springer Science Business Media. 1998.

[13] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia. 2011; 1(1): 98–106.

[14] Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, Omar AKM. Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering. 2013; 117(4): 426–436.

[15] Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju YH. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. Journal of Food and Drug Analysis. 2014; 22: 296–302.

[16] Khatun A, Rahman M, Akter A, Islam S, Akter M, Kabir S. Bioactivity of the bark of *Macaranga indica* Wight IC (Euphorbiaceae). World Journal of Pharmaceutical Research. 2014; 3(10): 172-182.

[17] Khan D, Mubashir M, Devidas Une H. Quantification of Quercetin Obtained from *Allium cepa* Lam. Leaves and its Effects on Streptozotocin-induced Diabetic Neuropathy. Pharmacognosy Res. 2017; 9(3): 287-293.

[18] Jain A, Katewa SS, Choudhary BL, Galav P. Folk herbal medicines used in birth control and sexual diseases by tribals of southern Rajasthan, India. J Ethnopharmacol, 2004; 90: 171–177.

[19] Yang D, Wang T, Long M, Li P. Quercetin: Its Main Pharmacological Activity and Potential Application in Clinical Medicine. Oxid Med Cell Longev. 2020;2020: 8825387. doi: 10.1155/2020/8825387.

[20] Das S, Mandal AK, Ghosh A, Panda S, Das N, Sarkar S. Nanoparticulated quercetin in combating age related cerebral oxidative injury. Current Aging Science. 2008;1(3):169–174.