ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

ANTIOXIDANT PROPERTY OF *ANDROGRAPHIS PANICULATA* AND *GYMNEMA SYLVESTRE*

B. Pouline*, Pawlin Vasanthi Joseph

Dept. of Zoology, Nirmala College for Women (Autonomous), Coimbatore, Tamilnadu, India

**Corresponding author: pouline617@gmail.com*

ABSTRACT

A study was conducted to estimate the antioxidant property of methanolic extracts of the selected herbal plants like *Andrographis paniculata* and *Gymnema sylvestre*. The antioxidant activity was evaluated by DPPH radical scavenging activity method. The *Gymnema sylvestre* methanolic extract showed more or less the same antioxidant activity by inhibiting DPPH than the methanolic extract of *Andrographis paniculata*.The quantitative analysis of flavonoids showed a value of 0.863mg/ gram in *Gymnema sylvestre* which was close to the standard value of 1.163 mg/ gram and *Andrographis paniculata* showed 0.545 mg/ gram of flavonoid content.The XRD analysis peaks may also be due to this high flavonoid content in the plant material. The study concludes that the antioxidant property may be due to the flavonoids present in the selected herbal plants.

Keywords: Antioxidant property, *Andrographis paniculata, Gymnema sylvestre*, DPPH, flavonoids, XRD analysis.

1. INTRODUCTION

Oxygen is essential for the survival of all species on this earth. During the process of oxygen utilization in physiological and metabolic process, approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric acid radicals. All the radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering each cell to face about 10,000 oxidative hits per second [1]. Recently interest has been increased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity [2]. Synthetic antioxidants such as butylated hydroxyl- toluene and butylated hydroxyanisole are currently used as food additives, and many species have similar antioxidant potentials as these synthetics [3]. Food industry uses natural antioxidants as a replacement of conventional synthetic antioxidants [4]. Antioxidants are inhibitors of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant materials act as radical scavengers and help in converting the radicals to less reactive species. A variety of free radical scavenging antioxidant is found in plants [5].

A few naturally occurring herbal plants contain many chemical components which have pharmaceutical property which influence the antioxidant enzymes and provide protection against free radical induced damages [6]. The herbal plants which show antioxidant and antimicrobial property have the potential to act as safer substitutes because of their molecular structure which is different from that of microbes and chemical based on pharmaceuticals which make their mode of action different [7].

Several medicinal plants including, *Andrographis paniculata* have been shown to exhibit antimicrobial and anti-oxidant characteristics due to the presence of various phytochemicals such as flavonoids, phenolic diterpenes, phenolic acids, tannis, carotenoids and tocopherols[8-12].

Andrographis paniculata, commonly known as 'King of Bitter', is a small, annual, branched and erect plant belonging to the family Acanthaceae. *Andrographis paniculata* Nees, It grows abundantly in south eastern Asia including India, Sri Lanka, Java, Pakistan, Indonesia and Malaysia. It prefers to grow well in a diversity of habitats such as moist, shady areas, hill slopes, plains, farms, seashores, waste lands and dry or wet lands [13]. The phyto- constituents of *Andrographis paniculata* plant which has been well elucidated include diterpenes, flavonoids, terpenoids, lactones, alkanes, ketones, aldehydes, andrographolides (the major component), paniculides,

polyphenols and several other sub- units of andrographolides [14,15]. The good free radical scavenging activity in *Andrographis paniculata* is due to the presence of flavonoids especially phenolic compounds [16].

Gymnema sylvestre also known as Gurmar, belongs to the family Asclepiadacea. The plant is a large tropical liana, native to central and Western India [17]. It is a woody, climbing herb grown in India, China, Indonesia, Japan, Malaysia, Srilanka, Vietnam and South Africa [18]. The leaves are utilized as anti-viral, diuretic, anti-allergic, hypoglycemic, hypolipidemic, for the treatment of obesity and dental caries [19]. It also acts as feeding deterrents to caterpillar, Prodeniaeridania [20] prevent dental caries caused by Streptococcus mutans (HijiYasutake, 1990) and in skin cosmetics [21]. It contains triterpenesaponins, quercitol, β- amyrin related glycosides and stigmasterol with pharmacological importance viz. antidiabetic [22]. Antimicrobial [23] and hepatoprotection [24].

2. MATERIAL AND METHODS

2.1. Collection of plant leaves

Fresh leaves of *Andrographis paniculata,* also known commonly as "Kings of Bitters," and *Gymnema sylvestre,* widely known as 'gurmar' were collected from Ooty, Nilgiris. Tamilnadu.

2.2. Preparation of plant powder

The collected leaves were washed; shade dried and powdered using pulverizer. The powdered leaves were sieved to remove the large (granules) fiber particles and used as plant additive for fish feed preparation.

2.3. Preparation of fish feed

Soybean meal (80gm) was taken in powder form as principal ingredient. Other ingredients like corn flour (20gm) were added and mixed well and turmeric (0.5gm) as antibiotic. The said mixture was boiled, cooled at room temperature. It was kept under refrigeration for 12 hours. After 12 hours it was squeezed over polythene sheet and dried at room temperature for 48 hours. The dried nodules are crushed into small pellets then pellets are sun dried to avoid fungal infection, weighed and stored in the bottle [25]. Eight experimental feeds were prepared by adding the plant additive (*A. paniculata* and *G. sylvestre*) at 0.5gm, 1gm, 1.5grms 1 and 2gms) separately and the feed without plant leaves is kept as control. The pellets were prepared by using domestic appliances with pore size 0.5mm diameter and stored for further use.

2.4. Experimental design

The laboratory experiments were laid in completely randomized design (CRD). Five replications for each treatment and control were maintained simultaneously. The experiment was conducted using 15 liter plastic troughs. The troughs were stocked with 10 fishes with mean initial weight of 20- 25 grams. The fishes were starved for a night prior to the experiment. The experiment was conducted for 30 days and fishes were fed with prepared control and experimental feed. The medium was changed daily in order to remove fecal and unconsumed feed.

2.5. Parameters taken for study

2.5.1. DPPH radical scavenging activity[26]
2.5.2. FTIR analysis[27]
2.5.3. XRD analysis(X- ray diffractometer)
2.5.4. Quantitative estimation of Flavonoids [28]

3. RESULTS AND DISCUSSION

3.1. DPPH radical scavenging activity

The antioxidant activity of *Andrographis paniculata* and *Gymnema sylvestre* was evaluated using DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay. Methanolic extracts obtained from the leaves of *Andrographis paniculata* and *Gymnema sylvestre* were used for the assay.

Table 1: **Anti-oxidant activity of methanolic extract of selected plants against standard**

Concentration (100µg/ ml)	*Andrographis paniculata*	*Gymnema Sylvestre*	Standard (Ascorbic acid) (0.1/ml)
20	15%	18%	22%
40	35%	38%	33%
60	52%	56%	58%
80	68%	73%	79%
100	84%	86%	91%

Table 1 shows the result of the free radical scavenging activity (DPPH) in % inhibition. The result revealed that the methanol fraction of *Gymnema sylvestre* exhibited the highest DPPH radical scavenging activity with 86% at 100 µg/ ml (which is nearly close to the value of Ascorbic acid i.e. 91%) followed by 73%, 56%, 35% and 18% at the concentrations of 80 µg/ ml, 60 µg/ ml , 40 µg/ ml and 20 µg/ ml respectively. While *Andrographis paniculata* showed highest activity of 84% at 100 µg/ ml concentration and the least DPPH radical scavenging activity was noted in *Andrographis paniculata* at 20 µg/ ml concentration (15%).

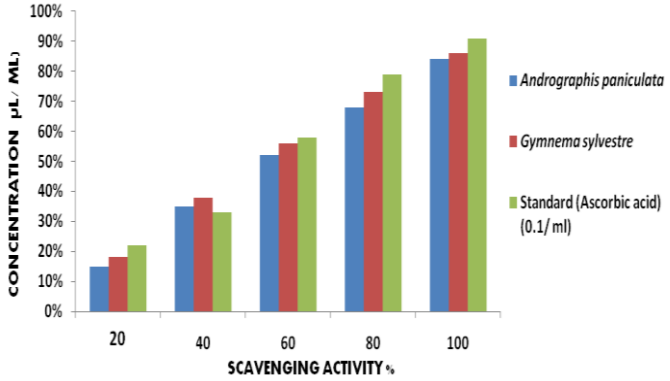

Fig.1: Anti-oxidant activity of methanolic extract of selected plants against standard

This result is in agreement with a study conducted by [29], who evaluated the phytochemical and in vitro antioxidant property of *Gymnema sylvestre* and reported significant antioxidant activity in ethanolic extract of Gymnema Sylvestre, this is because of the presence of preliminary phytochemicals such as tannins, saponins, phenols, flavonoids and alkaloids. Estimated the antioxidant property of methanol extracts of 12 Indian medicinal plants and the result showed seven extracts with 90% activity when compared with standards. When certain amount of interfering pigment was removed activity was increased [30]. Hence the free radical activity was due to the removal of some selective pigments. A powdered sample and methanolic extract of 11 medicinal plants were subjected to analysis of proximate composition and measurement of antioxidant activity. Result obtained indicated that the antioxidant potential varied from plant to plant. The poly phenolic constituents in the extracts were responsible for the radical scavenging activity [31].

3.2. FTIR analysis

The FTIR spectrum of leaf powder of *Andrographis paniculata* and *Gymnema sylvestre* are given in (Table- 2 and 3). The data on the peak values and the probable functional group were obtained by FTIR analysis.

Table 2: IR Absorption Frequencies of *Andrographis paniculata*

Functional group	Type of vibration	Characteristic absorption (cm^{-1})	Intensity
C-H	Stretch	2978.09	Strong
C=O	Stretch	1743.65	Strong
C=C	Stretch	1627.92	Variable
C-N	Stretch	1319.31	Medium-weak
C-O	Stretch	1242.16	Strong
C-O	Stretch	1026.13	Strong
C-Cl	Stretch	671.23	Strong
C-Cl	Stretch	601.79	Strong

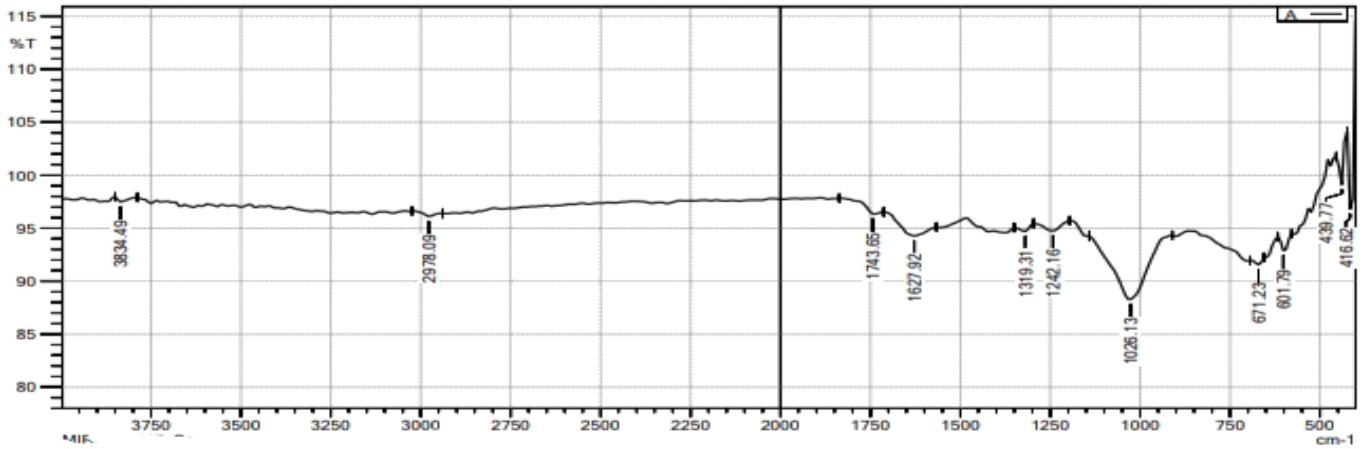

Fig. 2: IR Absorption Frequencies of *Andrographis paniculata*

Table 3: IR Absorption Frequencies of *Gymnema sylvestre*

Functional group	Type of vibration	Characteristic absorption (cm^{-1})	Intensity
O-H	(Stretch, H- bonded)	3302.13	Strong, broad
C-H	Stretch	2916.37	Strong
C-H	Stretch	2854.65	Strong
C=O	Stretch	1728.22	Strong
C=C	Stretch	1635.64	Variable
C=C	Stretch	1550.77	Medium-weak, multiple bands
C-F	Stretch	1381.03	Strong
C-F	Stretch	1319.31	Strong
C-F	Stretch	1242.16	Strong
C-F	Stretch	1026.13	Strong
C-Cl	Stretch	671.23	Strong
C-Cl	Stretch	601.79	Strong

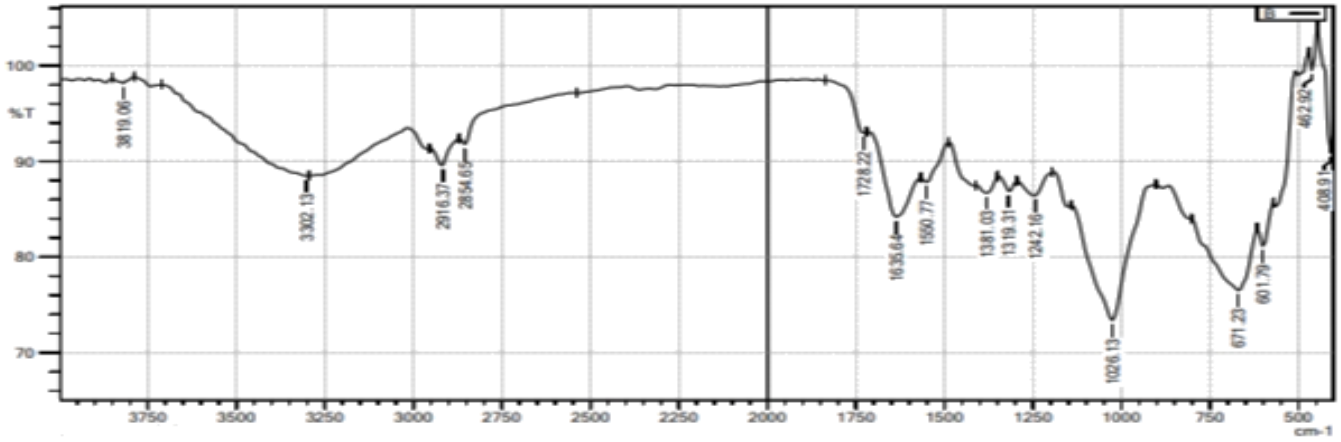

Fig. 3: IR Absorption Frequencies of *Gymnema sylvestre*

The result indicated that the leaf powder of *Andrographis paniculata* exhibited different characteristic band at 2978.09 cm^{-1}, 1743.65 cm^{-1}, 1627.92 cm^{-1}, 1319.31 cm^{-1}, 1242.16 cm^{-1}, 1026.13 cm^{-1}, 671.23 cm^{-1} and 601.79 cm^{-1}, indicating the presence of two alkane groups (C-H), one carbonyl group (C=O), one amine group (C-N), two ether groups (C-O), and two alkyl halide (C-Cl) groups. *Gymnema sylvestre* showed 12 different characteristic bands indicating the presence of alcohol group (O-H), two alkane groups (C-H), one carbonyl group (C=O), two aromatic groups (C=C), and six alkyl halide groups in which two C-Cl and four C-F groups.

This study is in agreement with the study carried out to estimate the FTIR and ED spectral analysis of plant parts like leaf, stem, and root of the medicinial plants and reported the presence of characteristic functional groups of carboxylic acids, amines, amides, sulphur dervatives, polysaccharides, nitratres, chlorates and carbohydrate that are responsible for various medicinial properties of the selected plants [32].The FTIR analysis of methanolic and aqueous leaf extracts of *Bauhinia racemosa* revealed the presence of protein, oil, fats, phenolic compounds, flavonoids, saponins, tannins and carbohydrate as major functional groups [33].

A study was conducted to screen the functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, halogens that are responsible for various medicinal properties of *Aervalanata* [34]. An analyzes on the ethanolic extracts of *Ichnocarpus frutescens*, by FTIR, revealed functional group components of amino acids, amides, amines, carboxylic acid,carbonyl compounds, organic hydrocarbons and halogens [35].

3.3.X- ray Diffraction analysis

X-ray diffraction analysis of *Andrographis paniculata* and *Gymnema sylvestre* showed the XRD pattern of the leaves obtained from colloid samples in (Figure 4 and 5 & Table 4 and 5). Three peaks were observed in *Andrographis paniculata* at 15.1403, 24.6201 and 38.3846, and only one peak was noted at 26.8981 in *Gymnema sylvestre*. Each of the plant extract has unique X - ray diffraction patterns that determine the measurement of crystallinity in the sample. The presence of crystals indicates the higher intensity peaks.

The result of the present study has been supported by an analysis made by the X- ray Diffraction of Cauliflower obtained from colloid samples and observed nine peaks at 11.660, 20.790, 21.490, 28.210, 29.170, 31.170, and 33.470, 40.670 and 43.500. These Braggs reflections clearly indicated the presence of 219.58, 299.22, 116.79, 49.92, 221.85, 184.02, 101, 33.21 and 48.52. Each of the plant extract has a unique X- ray diffraction pattern that determines the measurement of crystallinity in the sample. The presence of crystals indicates the higher intensity peaks [36].

Table 4: Peak list of *Andrographis paniculata* XRD

Pos. [°2 Th.]	Height [cts]	FWHM Left [°2 Th.]	d- spacing [Å]	Rel. Int. [%]
15.1403	80.64	0.8029	5.85195	80.90
24.6201	99.68	0.3346	3.61600	100.00
38.3846	55.86	0.8029	2.34513	56.04

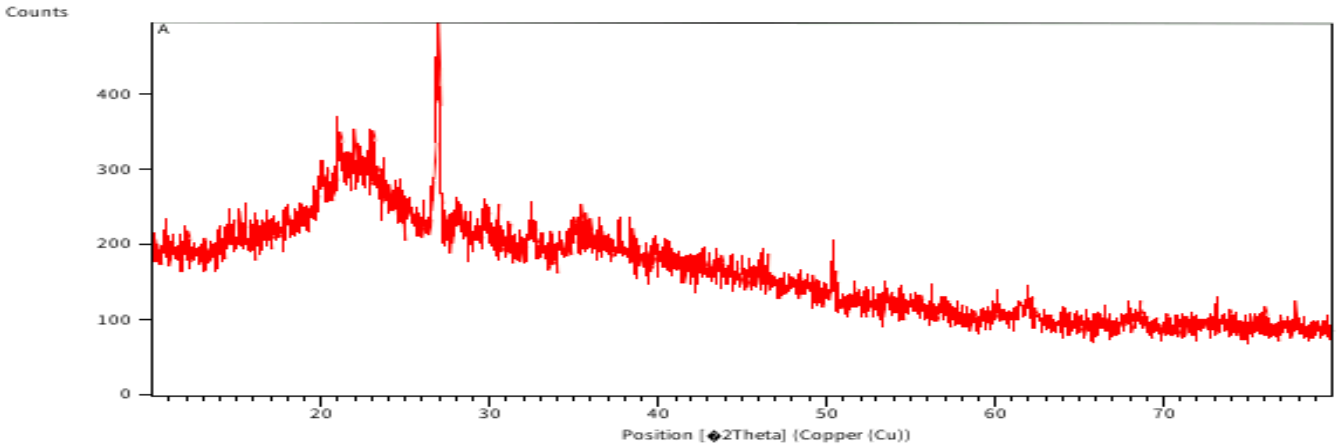

Fig. 4: Peak list of *Andrographis paniculata*

Table 5: Peak list of *Gymnema sylvestre* XRD

Pos. [°2 Th.]	Height [cts]	FWHM Left [°2 Th.]	d- spacing [Å]	Rel. Int. [%]
26.8981	213.71	0.3264	3.31196	100.00

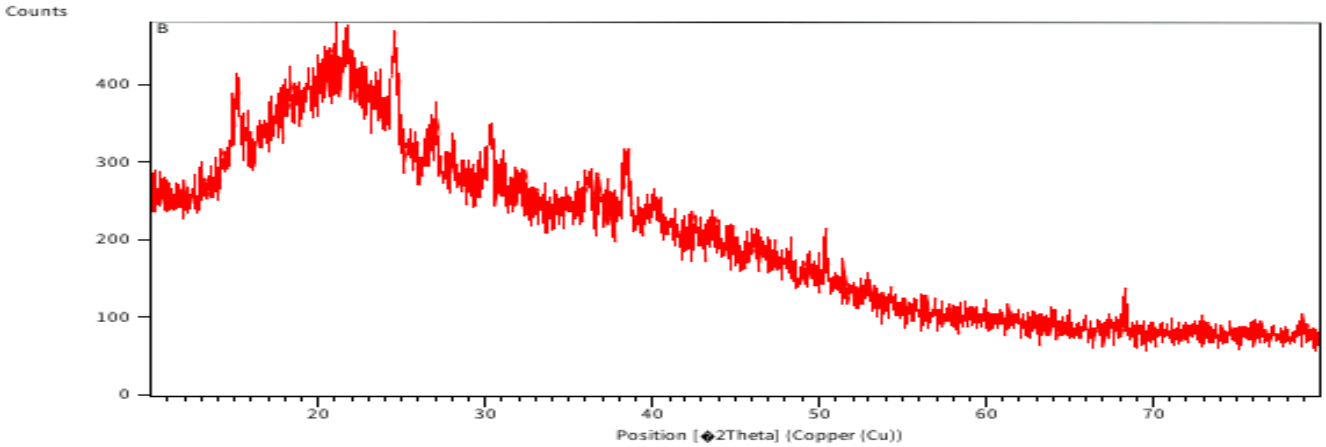

Fig. 5: Peak list of *Gymnema sylvestre*

3.4. Estimation of Flavonoids

The flavonoid content of the leaves in terms of quercetin equivalent (the standard curve equation: y = 0.0067× + 0.0132, r^2 = 0.999) were analysed. The flavonoid content was observed more similar with the standard in *Gymnema sylvestre* (0.863mg/ gram) which was close to the standard value of 1.163 mg/ gram. *Andrographis paniculata* showed 0.545 mg/ gram of flavonoid content.

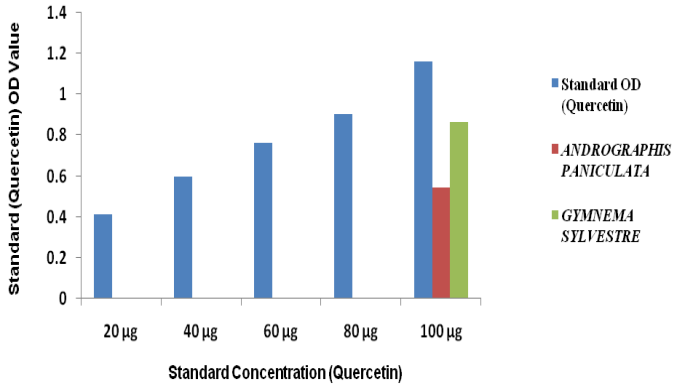

Fig. 6: Estimation of Flavonoids

Table 6: Quantitative Estimation of Flavonoids

Standard (Quercetin) Concentration	Standard (Quercetin) OD	*Andrographis paniculata*	*Gymnema sylvestre*
20 µg	0.412	0.545	0.863
40 µg	0.596		
60 µg	0.762		
80 µg	0.902		
100 µg	1.163		

4. CONCLUSION

The present study revealed that the methanolic leaf extract of *Gymnema sylvestre* showed high anti- oxidant property by inhibiting DPPH free radicals and exhibited that the leaves of *Gymnema sylvestre* is rich in different types of phytochemical constituents especially flavonoids. Since flavonoid, a phenolic compound, which usually exhibit very strong antioxidant property, may be the reason behind the selected plants showing high antioxidant property. The XRD analysis peaks may also be due to this high flavonoid content in the plant material. So, it is concluded that methanolic extracts of *Gymnema sylvestre* can be used as an accessible source of natural anti- oxidant agent.

5. REFERENCES

1. Mondal SK, Chakraborty G, Gupata M, Mazumder UK. *Indian Journal of Experimental Biology*, 2006; **44**:39-44.
2. Kumaran A, Karunakaran JR. LWT- *Food science and Technology*, 2007; **40(2):** 344-352.
3. Raad AK, Anoosh BH, Abbas MF, Javed A. *Journal of Pharmacognosy and Phytochemistry*, 2015; **4(1):**95-98.
4. Govindarajan R, Rastogi RS, Madhavan V, Shirwaikar A, et al. *Biol. Pharm. Bull*, 2003; **26(10):**1424-1427.
5. Mandal S, Yadav S, Yadav S, Nema RK. *J Chem Pharm Res*, 2009; **1(1):**102-104.
6. Aruldoss K, Kannan R, Chandira A, Samipillai SS. *International Journal of Modern Research and Reviews*, 2014; **2(5):**171-177.
7. Bera D, Lahiri D, Nag A. *Journal of Food Engineering,* 2006; **74**:542-545.
8. Akowuah GA, Zhari I, Norhayati I, Sadikun, Khamsah SM, Sinensetin. *Food Chemistry,* 2004; **87**:559-566.
9. Akowuah GA, Ismail Z, Norhayati I, Sadikun A. *Food Chemistry,* 2005; **93**:311-317.
10. Ebrahimabadi AH, Ebrahimabadi EH, Djafari-Bidgoli Z, Kashi FJ et al. *Food Chemistry,* 2010; **119**:452-458.
11. Edziri HL, Smach MA, Ammar S, Mahjoub MA et al. *Industrial Crop Production,* 2011; **34**:1182-1185.
12. HijiYasutake US. Gymnemic acid for prevention of dental caries. *In: Chemical Abst*, 1990; **113**:463-64b.
13. Prajapati ND, Purohit SS, Sharma AK, Kumar T; *A handbook of Medicinal Plants*, Jodhpur; Agrobios; 2003; 352-353.
14. Niranjan A, Tewari SK, Lehri A, *Indian Journal of Natural Product Resources*, 2010; 125-135.
15. Akbar S,*Andrographispaniculata*: *Alternative Medicine Review*,2011; **16(1):**66- 77.
16. Priyanka D, Alok, KS. *International Journal of Innovative Research in Science, Engineering and Technology*, 2014; 03:15175-15184.
17. Stoecklin W, *J Agric Food Chem*, 1969; **17(4):**704-708.

18. Hill AF. Economic Botany. A textbook of useful plants and plant products. 2 edn. McGarw-Hill Book Company Inc; New York; 1952.
19. Porchezhian E, Dobriyal RM, *Pharmazie*, 2003; **58(1):**5-12.
20. Granich SM, Halperin BP, Eisner T. *J. Insec. Physiol*, 1974; **20**:435-439.
21. Komalavalli N, Rao MV, *Plant Cell. Tissue Organ Cult*, 2000; **61**:97-105.
22. Gopi C, Vatsala TM. *African Journal of Biotechnology,* 2006; **5**:15-19.
23. Zargar M, Azizah AH, Roheeyati AM, Fatimah AB, et al. *Journal of Medicinal Plants Research*, 2011; **5**:2525-2532.
24. Rana AC, Avadhoot Y. *Arch Pharm Res,* 1991; **14**:93-95.
25. Bhosale SV, Bhilave MP,Nadaf SB. *Research Journal of Agricultural Sciences*, 2010; **1(3):**284-287.
26. Braca A, Tommasi ND, Bari LD. *J. Nat Pro*, 2001; **64**:892-895.
27. Ashokkumar R, Ramaswamy M, *Int. J. Curr. Microbiol. App. Sci,* 2014; **3(1):**395-406.
28. Patel S, Patel J, Patel, RK. *Int J Pharm Tech Res.*, 2012; **4(4):**1520-1526.
29. Kalyani S, Bandita D, *Journal of Medicinal Plants Studies*, 2014; **2(4):**19-23.
30. Jadhav HR, Bhutani KK, *National Institute of Pharmaceutical Education and Research*, 2002; **16(8):**771-773.
31. Nilima SR, Sunil MH, *Indian Journal of Pharmaceutical Sciences*, 2011; **73(2):**146-51.
32. Muruganantham S, Anbalagan G, Ramamurthy N, *Romanian Journal of Biophysics*, 2009; **19(4):**285-294.
33. Gaurav Kumar L, Karthik, Bhaskara Rao KV, *International Journal of Pharmaceutical Sciences and Research*, 2010; **1(11):**51-58.
34. Ragavendran P, Sophia D, Arul Raj C, Gopalakrishnan VK. *Pharmacologyonline*, 2011; **1**:358-364.
35. Thangarajan S, Paramasivam R, Chinthamony AR, Palanisamy CP, et al. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2012; **4(5):**343-345.
36. Gowri G, Manimegalai K. *World Journal of Pharmacy and Pharmaceutical Sciences*, 2017; **6(7):**1277-1282.