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Phytoconstituents and Comprehensive evaluation of Antioxidant activity and *In-vitro* Antimicrobial properties of *Ageratum conyzoides* plant

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Abstract

Ageratum conyzoides is used as a medicinal plant all around the world. The objectives of current work is to study the phytochemicals screening of leaves extracts of *Ageratum conyzoides* by using solvent as isopropyl alcohol and water to revealed the presence of various secondary metabolites. The different extracts of leaves were further carried out for the isolation and purification of compounds through the different column chromatography. The characterization of isolated compounds was carried out by using Infra-red spectroscopy. The effluents of extract were evaluated for the antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay The DPPH radical scavenging activity of *Ageratum conyzoides* extract with ascorbic acid as reference, the extracts were also taken to investigate the in-vitro antibacterial activity by using agar disc diffusion method.

Keywords: *Ageratum conyzoides,* Phytochemical screening, Characterizations, Antioxidant activity, Antibacterial activity.

1. Introduction

The word "ageratum" comes from the Greek word "a geras," which means "non-aging" and refers to the longevity of the entire plant. Conversely, the word Conyzoides is derived from the Greek name Inula helenium, which is similar to the plant [1]. The plant is a member of the Eupatorieae tribe of the Asteraceae family. There are about thirty species in the genus *Ageratum*, although only a few have been studied phyto-chemically [2]. This tropical plant is widely distributed in South America, certain regions of Asia, Australia, and West Africa. It is an aromatic, erect, branching, slender, hairy, annual herb that reaches a height of about one meter, with white hairs covering the stems and leaves. The leaves are stalked, ovate, 4–10 cm long and 1–5 cm wide, with somewhat pointed tips and bases and

long, round-toothed margins. Less than 6 mm in diameter, the purple to white flowers are grouped in near terminal. The seeds are photoblastic and frequently disappear after a year, but the black fruit is easily disseminated [3]. The plant typically grows in trash and on abandoned buildings. It gets its name from its unusual smell, which Australians have compared to that of a male goat Marijuana, often known as billy goat weed. Because it contains coumarin and HCN, the essential oil extracted from the plant has been proven to be harmful to rabbits and to have a strong, sickening smell [4].[5] The herb is only consumed by humans for therapeutic purposes; nevertheless, in certain cultures, it is considered a delicacy and is fed to domestic guinea pigs, horses, and cats [6].[7] Pyrrolidine alkaloids are known to be hepatotoxic and to induce lung cancer and other illnesses[8], but because of their connection to leptidoptera, they are of biological interest.[9].

Many regions of Asia, Africa, and South America have employed *Ageratum conyzoides* for medicinal purposes [10]. The plant has been widely used to treat dyspnea, headaches, mental and infectious disorders, skin conditions, and wound healing [12, 13]. Utilised in conventional medicine for its anti-asthmatic, antispasmodic, and hemostatic properties, as well as for uterine issues, pneumonia, and other conditions by applying them to the patient's chest [15]. It is used as an oil lotion for purulent ophthalmia and as a leprosy treatment in India. [16] For stomach illnesses such diarrhoea, dysentery, intestinal colic, ß atulence, rheumatism, and fever, the herb is taken as a decoction or infusion [17].

Figure 1: *Ageratum conyzoides* plant

In the current work we have studied the phytoconstituents and comprehensive evaluation of antioxident activity and in-vitro antimicrobial properties of ageratum conyzoides plant.

2. Methodology

Collection of Sample

The fresh leaves of *Ageratum conyzoides* were collected from Melghat Forest Near Madki Village, Tal-Chikhaldara, Dist- Amravati (Central region of India) in the month of December – 2022, were authenticated by a taxonomist from Department of Botany, Smt. Narsamma A.C.S. College, Kiran Nagar, Amravati.

Processing of the sample

Fresh Leaves of *Ageratum conyzoides* plant was washed well by using tap water and thrice using distilled water. It was dried under the shade for a period of 10-15 days, at an ambient temperature of 33˚C. After drying, the plant Materials were cut into small pieces. The dried samples were grinded properly by using a mortar and pestle. Then it was grind well by using a mixture grinder, to obtain a fine powder. Then it was stored at room temperature in an air tied container.

Preparation of extracts

Dried powdered material (50 gm) of *Ageratum conyzoides* leaves was extracted with isopropyl alcohol and distilled water separately in soxhlet apparatus. The temperature of heating mantle was adjusted to 83ºC for isopropyl alcohol extraction, while 100ºC for aqueous extraction. The extracts were concentrated by gradually evaporating the respective solvents on hot water bath. The concentrated extract was collected in air tied sterile bottles and kept it in refrigerator.

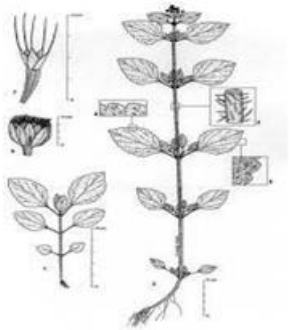


Figure 2:

Preliminary phytochemical investigation

The major secondary metabolites classes such as Flavonoids, alkaloids, Tannins, Saponins, Phenolic Compounds, Cumarine and Terpenoids were screened in *Ageratum conyzoides* plant according to the common Phyto-chemical methods described by Harbone [18], Sofowara [19] Kokate [20].

Test for Tannins

About 500 mg of the plant extract was boiled with 10 mL of distilled water; and 0.1% Ferric chloride was added to the mixture; then the indication of blue-black coloration shows the presence of tannins.

Test for Alkaloids

The plant extract was dissolved in 100 mL of water, filtered, and cooked in steam with 5 mL of the filtrate and three drops of 1.0 % HCl. Then, 1.0 mL of the heated mixture was combined with 6 mL of the Mayer-Wagner reagent. The appearance of a cream or brown-red colored precipitate indicated the presence of alkaloids.

Test for Saponins

About 1.0 ml of the extract and 5 mL of distilled water were combined and agitated. Then, the formation of foam confirmed the presence of saponins.

Test for Flavonoids and Glycosides

100 mg of the plant extract was mixed with 20 mL of ethanol and filtrated. 2.0 mL of the filtrate, concentrated HCl and magnesium ribbon were mixed. The formation of a pink or red color indicates the presence of flavonoids. Adding 1.0 mL of distilled water and NaOH to 0.5 mL of crude extract, the formation of a yellowish color indicated the presence of glycosides.

Test for Steroids

About 1 mL of the crude extract was combined with 10 mL of chloroform and 10 mL of sulfuric acid, and the formation of the bilayer (red top layer and greenish bottom layer) reveals the presence of steroids.

Test for Terpenoids

The presence of terpenoids was determined by the formation of a reddish-brown color in the test for terpenoids, which included mixing of 0.5 mL of crude extract with 2 mL of chloroform and 3 mL of sulfuric acid.

Test for Phenols

About 1 mL of the extract was combined with three drops of $FeCl_3$, and 1 mL of K_2Fe (CN_6). The formation of greenish- blue forms confirmed the presence of phenols.

Antioxidant activity of *Ageratum conyzoides* plant

Sample Preparation

The concentrated isopropyl alcoholic extract was transfer to various Petri dishes for evaporating the isopropyl alcohol. Evaporation is done at ambient temperature and dried powder was collected in sterilized eppendorf tube and stored at 5 ^{0}C for further use. The stock solution of crude extracts was prepared by dissolving a known amount of dry extract in 98% isopropyl alcohol. The working solutions (1000, 2000, 3000, 4000, 5000 µg/ml) of the extracts were prepared from the stock solution using suitable dilution.

DPPH free radical scavenging activity of isopropyl alcoholic extract

The free radical scavenging activity of the isopropyl alcoholic extracts, based on the scavenging activity of the stable 2, 2- diphenyl-1- picrylhydrazyl (DPPH) free radical was determined by using the method described by "Fully Automated Spectrometric Protocols for Determination of Antioxidant Activity: Advantages and Disadvantages".

The diluted solutions of the test extracts were prepared in isopropyl alcohol. Ascorbic acid was used as standard in 1000-5000 µg/ml solution. 4.94 mg of DPPH was prepared in 100 ml isopropyl alcohol and 3.96 ml of this solution was mixed with 50 µl of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 520 nm using UV-Vis Spectrophotometer (UV-1700 Shimadzu). DPPH solution was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below.

% of DPPH radical scavenging activity (%RSA) = [(A control-A sample) /A control] ×100

$Abs_{control}$ is the absorbance of DPPH radical + isopropyl alcohol: Abs_{sample} is the absorbance of DPPH radical + Sample extract.

The measurements were performed in triplicate. Absorbance values were corrected for free radical decay using blank solutions. The IC_{50} (Concentration providing 50% inhibition) was calculated graphically using calibration curve verses‟ percentage of inhibition.

Antimicrobial activity of *Ageratum conyzoides* plant

Sample Preparation

The concentrated isopropyl alcoholic extract was transfer to various Petri dishes for evaporation the isopropyl alcohol. Evaporation was done at ambient temperature and dried powder was mixed with ethyl acetate solution. These ethyl acetate plants extract was further used for antibacterial activity.

Test Bacteria

Staphylococcus aureus (ATCC-33591), *Escherichia coli* (ATCC-14948), *Klebsiella pneumonia* (MTCC- 4030), *Salmonella typhi* (ATCC-25812),were purchased from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India and used for assessment of antibacterial activity.

Antibacterial screening

Agar disc diffusion assay was utilized to assess the bactericidal activity of different antibiotics against test strains of E. Coli, K. pneumonia, S. aureus, and S. typhi, following the guidelines outlined in the Manual of Antimicrobial Susceptibility Testing (Cavalieri et al., 2005). The collected microorganism strains were added to a conical flask that held 100 millilitres of nutritional broth. These conical flasks, known as "seeded broth," were incubated for twenty-four hours at 37 0C. Muller Hinton Agar (Himedia, Mumbai, India) was used to make the media, which were then put onto Petri dishes and infected using cotton swabs with the test organisms from the seeded broth. After being impregnated with 20 µl of test extract, sterile discs of six millimetres in width were placed on top of the seeded agar plate. The plates were kept at 37 0C for the entire night. By measuring the inhibitory zone that developed around the discs, antibacterial activity was determined. Three runs of the experiment were conducted, and the mean values were reported. The standard utilised was 10 µg/disc of Tetracycline.

Results and Discussion

The phytoconstituents of leaf extract of *Ageratum conyzoides* was screened by using two different solvents like Water and Isopropyl alcohol. Phytochemical analysis of the plants revealed some difference in the phytoconstituents of two extracts.The phytochemical screening of leaf extracts of *Ageratum conyzoides* in isopropyl alcoholic medium revealed the presence of rich qualitative presence of some important secondary metabolites like Carbohydrates, Flavonoids, Tannins, Saponins, Alkaloids, Terpanoids, Steroids etc. whereas in aqueous medium leaf extracts was good in Phytochemical activity revealed the presence of Carbohydrates, Tannins, Proteins, Cumarine, Phenolic compounds etc, as shown in Table 1.

Numerous active phytochemicals have been shown to have a variety of functions, which may aid in the prevention of terminal illnesses. Anti-inflammatory properties are found in biochemicals and phytochemicals, including tannins, alkaloids, terpenoids, flavonoids, saponins, and steroids [21,22]. According to Oliver [23] and Cherian et al. [24], many cardiac glycosides, flavonoids, tannins, and alkaloids exhibit hypoglycemic properties. Studies on animals have also demonstrated that the mono, di, and triterpenoids lower blood sugar levels [25]. Analgesic effects were demonstrated by triterpenoids and high molecular weight steroids [26, 27]. Activities of the central nervous system are also caused by the steroids and saponins [28].

Using the stable free radical DPPH (deep purple colour), which has the benefit of not being impacted by any side reactions, the radical scavenging ability of the Ageratum conyzoides isopropyl alcoholic leaf extract was evaluated.

Table 1. Phytochemical analysis of *Ageratum conyzoides*

Sr. No.	Phyto-chemical	Test performed	Aqueous extract	Isopropyl alcoholic extract
			Leaf	Leaf
1	Carbohydrates	Molisch Test	++	+
2	Sugar	Benedict Test	+	-
3	Protein	Xanthoproteic Test	-	-
4	Tannins	Gelatin Test	+	+
5	Phenolic comp	Lead Acetate Test	++	++
6	Phytosterols	Libermann Burchard Test	-	++
7	Steroids	Ring Test	-	+
8	Amino acids	Ninhydrin Test	-	-
9	Flavonoids	Ethyl acetate Test	-	++
10	Terpenoids	Salkowski Test	-	++
11	Alkaloids	Dragendorff's Test	-	++
12	Anthraquinone	Borntrager‟s Test	-	-
13	Cumarine	Fluorescence test	+	+
14	Phlobatinins	Spot Test	+	-
15	Anthrol Glycosides	Borntrager‟s test	-	-
16	Cardiac Glycosides	Legal‟s test	+	++
17	Saponins	Foam Test	-	++
18	Fixed oils and lipids	Spot Test	+	+

Table 2. All Potential Bands, Corresponding Functional Groups, and Possible Compounds Identified in the Isopropyl alcohol Extract of the Formulation Using FT-IR Spectroscopy

Sr. No.	Frequency (cm^{-1})	Intensity	Band Assignments	Possible Compounds	Literature value (cm^{-1})
1	3625.05	B	H-O (stretching)	Alcohol	3650-3200
2	3291.59	M	Ar-N-H	Amines	3500-3300
3	3087.99	M	Ar-C-H	Aromatic H	3350-3000
4	1669.93	S	Ar-C=C	Benzene rings	1650-1550
5	1599.43	S	Ar C=N	Cynide, Imine	1600-1500
6	1893.73	S	C=O	Aldehyde, Ketone	1900-1700
7	1305.24	M	C-C	Alkane,	1400-1300
8	1247.33	M	C-N	Amines , Nitrile	1300-1250
9	1119.14	S	C-O	Alkyl, Aryl, Ether	1250-1100
10	937.49	S	para di substituted	Di substitution	990-940
11	764.79	S	Ortho di substituted	Di substitution	770-735

Table 3. Antibacterial activity of *Ageratum conyzoides* Ethyl acetate extract

Test Organism	Extract	Antibiotic (Tetracycline)	Antibiotic + Extract	Ethyl acetate (Control)
S. aur	20	35	35	0
P. aeru	18	14	14	0
P. acne	16	18	19	0
E. coli	10	17	17	0
K. pneu	10	19	18	0
S. typhi	00	14	14	0
Zone of inhibition in Diameter (mm)				

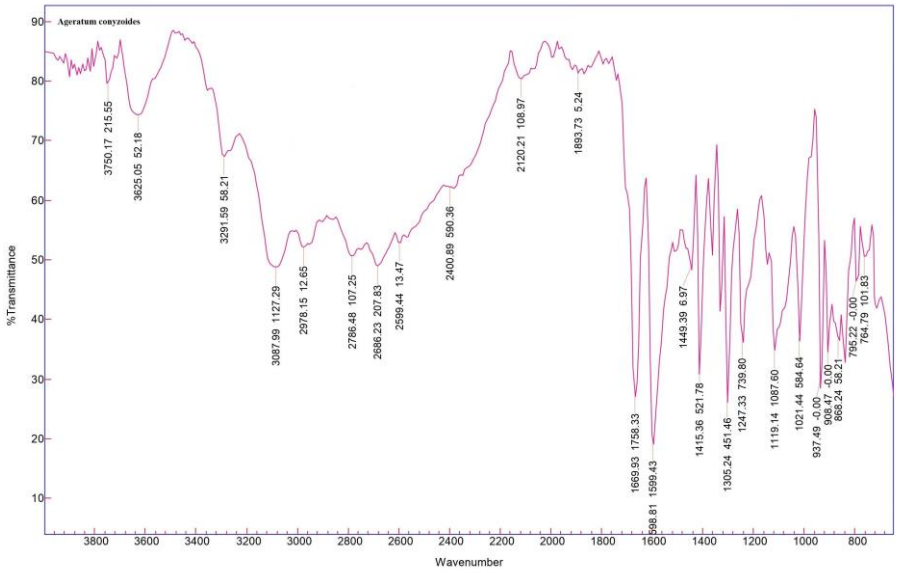
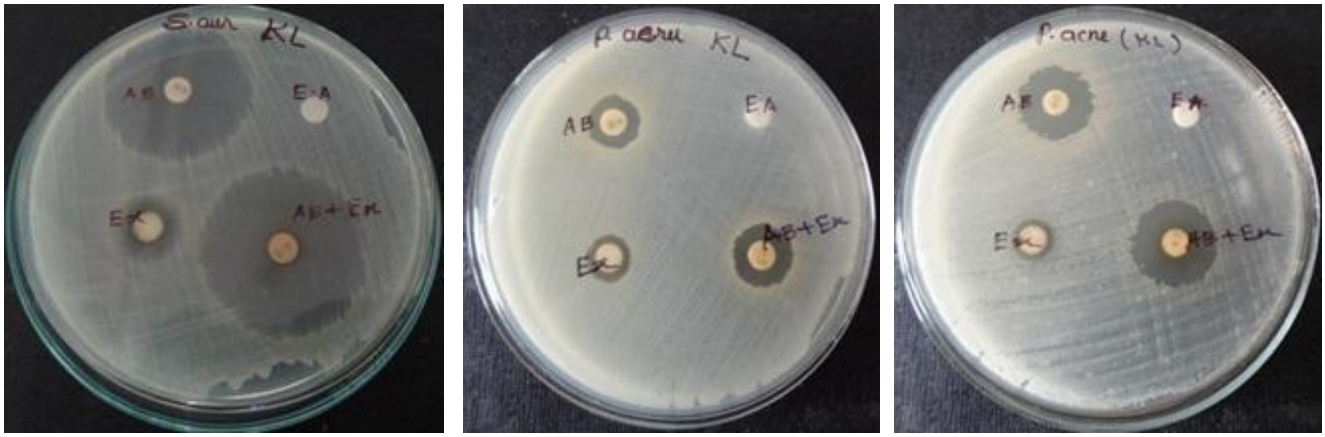


Figure 2: FT-IR spectrum representing potential bands in the Isopropyl alcohol extracts of the formulation.

E.A- Sterile disk(control), EX- Extract disk ,AB+ EX- Antibiotic + Extract disk



Staphylococcus aureus ***Pseudomonas aeruginosa*** ***Propionibacterium acnes***

Escherichia coli ***Klebsiella pneumonia*** ***Salmonella typhi***

Figure 4. Antibacterial activity of *Ageratum conyzoides* Leaf against six bacteria, in each image: AB- Antibiotic disk
++ indicates: strong presence, + indicates: weak presence, - indicates: strong absence

The leaf extract of Ageratum conyzoides (IC50 = 14135 µg/ml) was found to have a lower IC50 value than the standard ascorbic acid (IC50 = 2.816 µg/ml) in Figure 2, which displays the DPPH radical scavenging activity of Ageratum conyzoides isopropyl alcoholic extracts with ascorbic acid as reference. The phytoconstituents in plants, flavonoids and tannins, are what scavenge free radicals without any help. According to Saxena et al. [29], plants that contain flavonoids and tannins are considered to be rich sources of phenolic compounds, which function as primary antioxidants or scavengers of free radicals." According to Yamaguchi et al. [30], the antioxidants in the extract thus neutralise the DPPH free radicals (either by supplying a hydrogen atom or by electron transfer, possibly through a free radical attack on the DPPH molecule) and transform them into a colourless product (2, 2-diphenyl-1-picrylhydrazyl, or a substituted analogous hydrazine), which causes the absorbance at 517 nm to decrease.

Table 2 provides the plant extract's antibacterial properties against the six strains of bacteria that were investigated. All six organisms were effectively inhibited by the ethyl acetate leaf extract of Ageratum conyzoides. *P. aeru, P. acne, E. Coli*, and *S. aureus* showed the strongest inhibition. Because they included a variety of phenolic chemicals, the Ageratum conyzoides ethyl acetate extracts demonstrated strong antibacterial and antioxidant activities.

Conclusion

Ageratum conyzoides has been subjected to preliminary screening of phyto-constituents which indicates the presence of phyto-chemical compounds like Flavonoids, Alkaloids, Terpaenoids, Steroids, and phenolic compounds in the isopropyl alcohol extract of plant leaves. The spectroscopic study like FT-IR revealed the presence of functional groups such as aromatic primary amine, Aldehydic and Ketonic groups, aromatic cynide compound, and Alcoholic groups. Also it reveals the presence of Di and tri substituted aromatic rings.

The study concluded that the isopropyl alcoholic extracts shows significant antioxidant activities in a concentration dependent manner. The most useful parameter of extract was study on antibacterial activity against the gram positive and gram negative bacterial strains like *Staphylococcus aureus, Pseudomonas aeruginosa, Propionibacterium acnes, Escherichia coli, Klebsiella pneumonia* and *Salmonella typhi* in which *S. aureus, P. aeruginosa, P .acnes* showed better activity whereas *E. coli,* and *K. pneumonia* showed moderate activity and *S. typhi* did not show activity against the plant leaves extract.

Hence the plant contains potential antibacterial constituents that may be useful for evolution of pharmaceutical therapy for the ailments and treatment of infections caused by the strains of the test bacterial organisms, emphasizing socio-economic significance of *Ageratum conyzoides.*

Conflicts of interest: The author stated that no conflicts of interest.

References

1. Kissmann G, Groth D. Plantas infestantes e nocivas. Sau Paulo: Basf Brasileira; 1993.
2. Burkill HM. The Useful Plants of West Tropical Africa, vol. 1. Kew: Royal Botanic Gardens; 1985.
3. Marks MK, Nwachuku AC. Seed bank characteristics on a group of tropical weeds. Weed Res, 1986;26:151-7.
4. Sood VK. Chemical examination of the ß ower oil of *Ageratum conyzoides* L. Flav Ind, 1973;4:77.
5. Abbiw DK. Useful plants of Ghana, Intermediate Tech. Publication. London: Royal Botanic Gardens, Kew; 1990. p. 207.
6. Dalziel JM. The useful plants of West Africa. London: Crown Agents; 1937.
7. Menut C, Lamaty G, Amvam PH. Aromatic plants of tropical central Africa part X: Chemical composition of essential oil of *Ageratum houstonianum*. Flav Frag J 1993;8:1.
8. Couett CE, Crews C, Hanley BA. Analysis, separation and bioassay of pyrrolizidine alkaloids from comfrey. Nat Toxins 1996;4:1605-10.
9. Vera R. Chemical compostion of essential oil of *Ageratum conyzoides* L. from Reunion. Flav Fragr J 1993;8:257.
10. Githens TS. Drug plants of Africa. Afr Handbooks 1948; 8:59.
11. Kerharo J, Adam JG. La pharmacopee senegalaise traditionelle: Plantes. Medicinales toxiques vol. 1, Paris: Vigot; 1974. p. 101.

12. Adjanohoun EJ, Ahyi AM, Ake-Assi L. Medicine traditionelle et pharmacopee, 1988.
13. Durodola JI. Antibacterial property of crude extracts from a herbal wound healing remedy *Ageratum conyzoides* L. Planta Medica 1977;32:388-90.
14. Kokwaro JO. Medicinal plants of East Africa. Nairobi: East African Literature Bureau; 1976. p. 58.
15. Okunade AL. Ph.D Thesis, Nigeria: University of Ibadan; 1981. p. 84.
16. Katsuri TR, Thomas M, Abraham EM. Essential oil of *Ageratum conyzoides*: Isolation and structure of two new constituents. Indian J Chem 1973;11:91-5.
17. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: NISCIR; 2002. p. 9.
18. Harborne JB. Phytochemical Methods, London Chapman and Hall Ltd., 1973; Pp. 49-188.
19. Sofowora A. *Medicinal Plants and Traditional Medicinal in Africa.* 2nd Ed. Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd; 1993. Screening Plants for Bioactive Agents; pp. 134–156.
20. Kokate CK. Practical Pharmacognosy, 2001. Nirali Prakasham, Mumbai, India.
21. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals, *American Journal of Clinical Nutrition*, 78; 2003, pp. 517S-520S.
22. Manach C, Regerat F, Texier O. Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids, Nutrition Research, 16 (1996), pp. 517-544.
23. Oliver B. Journal of Ethnopharmacology, 2 (1980), pp. 119-127.
24. Cherian S and Augusti KT. Indian Journal of Experimental Biology, 33 (1995), pp. 608-611.
25. Luo J, Cheung J, Yevich E. Journal of Pharmacology and Experimental Therapeutics, 288 (1999), pp. 529-534.
26. Malairajan P, Gopalakrishnan G, Narasimhan S, Veni KJK. Journal of Ethnopharmacology, 19 (2006), pp. 425-428.
27. Sayyah M, Hadidi N, Kamalinejad M. Journal of Ethnopharmacology, 92 (2004), pp. 325-329.
28. Argal A and Pathak AK. Journal of Ethnopharmacology, 106 (2006), pp. 142-145.
29. Saxena M, Saxena J, Pradhan A. Flavonoids and Phenolic acids as antioxidants in plants and human health, Int. J. Pharm. Sci. Rev. Res., 2012; 16(2),: 130-134.
30. Yamaguchi T., Takamura H., Matoba T., Terao J. HPLC Method for Evaluation of the Free Radical-scavenging Activity of Foods by Using 1,1-Diphenyl-2-picrylhydrazyl. *Biosci. Biotechnol. Biochem.* 1998;62:1201–1204. doi: 10.1271/bbb.62.1201.