The Scientific Temper (2023) Vol. 14 (2): 484-489

E-ISSN: 2231-6396, ISSN: 0976-8653

Doi: 10.58414/SCIENTIFICTEMPER.2023.14.2.38

https://scientifictemper.com/

RESEARCH ARTICLE

Assessment of antioxidant activity and phytochemical screening in leaf extract of *Andrographis paniculate* (Burm. f.) nees

Anju Bhatnagar

Abstract

Fresh *Andrographis paniculata* leaves were collected from a local herbal garden, Dehradun and leaves extract was prepared in methanol and ethanol solvent. The free radical scavenging activity of *A. paniculata* extract against 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and and hydrogen peroxide (H_2O_2) radical was determined with UV-vis spectrophotometer. The IC_{50} value of methanolic and ethanolic extract of the plant in DPPH was 394.81 and 401.46 µg/mL, while in vitamin C was 285.69 µg/mL. However, the IC_{50} value of the hydrogen peroxide (H_2O_2) radical for methanolic extract was higher, 399.52 µg/mL than for ethanolic extract, 412.95 µg/mL. In conclusion, methanolic plant extracts were most potent against 50% inhibition and IC_{50} value and showed a good correlation with total phenolics content. Phyto-chemical analysis of the plant shows the presence of alkaloids, phenols, amino acids, flavonoids, saponins, steroids, and tannins. *A. paniculata* has strong antioxidant potential and contains a range of phytochemicals. Therefore, its effectiveness as a medical plant is due to the presence of various phenolics and antioxidant compounds in the plant.

Keywords: *Andrographis paniculata*, Antioxidant, DPPH, H_2O_2, Phytochemicals.

Introduction

Medicinal plants are a potential source of bio-molecules that are used as a phytomedicine (plant-based drug) for the treatment of various diseases and disorders for centuries (Joshi *et al*., 2016, Khajuria *et al*., 2021). The plant contains numerous bioactive components that can scavenge free radicals (Kalaiselvan *et al*., 2012). It may be essential to consume natural oxidants to enhance a weakened immune system (Saxena *et al*., 1998). The antioxidants protect the body from free radical-induced oxidative stress and help to prevent cancer and heart disease (Alok, *et al*., 2014).

Andrographis paniculata Nees is a medicinal plant belonging to the family Acanthaceae. This plant, known as Kalmegh, or 'King of Bitter' in English, grows abundantly in Southeast Asia, India, and Sri Lanka. The plant is widely used in Indian traditional medicine, including Ayurveda, Unani, and Siddha, as a home cure for many illnesses. Due to its significant anti-inflammatory characteristics, this plant is widely recognized for its curative properties against upper respiratory tract infections (Liu *et al*., 2008). *A. paniculata* shows a variety of biological properties, such as antibacterial, antiviral, cold and fever, anticancer, urinary tract infection, anti-diabetic, cardiovascular, immune-modulatory, and antihepatotoxic (Bhatnagar, 2023) etc.

The main active ingredient in the plant is andrographolide ($C_{20}H_{30}O_5$) (Chander *et al*.,1995). It is a diterpene lactone that gives the plant a bitter taste. Many research studies have focused on the anti-inflammatory (Chakravarti *et al*., 1951), hepatoprotective (Qin *et al*., 2006), anticancer (Handa *et al*., 1990), analgesic, and antipyretic (Lee *et al*., 2015) nature of andrographolide. The other main diterpenoids of *A. paniculata* are deoxy andrographolide, neoandrographolide, and 14-deoxy-11,12-didehydroandrographolide, deoxyandrographolide19β-D-glucoside, and flavonoids are 5,7,2′,3′-tetramethoxyflavanone and 5-hydroxy-7,2′,3′-trimethoxyflavone (Bhatnagar, 2023). Therefore, the study aims to determine the phytochemical analysis and antioxidant activity of *A. paniculata* in two different solvents in the quest to strengthen scientific knowledge.

Materials and Method

The plant leaves were obtained from the local herbal garden, in Dehradun (Uttarakhand), India. The identification and

Department of Chemistry, D.B.S. College, Dehradun, Uttarakhand, India.

***Corresponding Author:** Anju Bhatnagar, Department of Chemistry, D.B.S. College, Dehradun, Uttarakhand, India, E-Mail: dbsanju2014@gmail.com

How to cite this article: Bhatnagar, A. (2023).Assessment of antioxidant activity and phytochemical screening in leaf extract of *Andrographis paniculate* (Burm. f.) nees. The Scientific Temper, **14**(2):484-489.

Doi: 10.58414/SCIENTIFICTEMPER.2023.14.2.38

Source of support: Nil

Conflict of interest: None.

Received: 10/04/2023 **Accepted:** 20/05/2023 **Published** : 30/06/2023

authentication of the plant were done and the specimen was kept in the herbarium. The leaves were air-dried at room temperature and ground into a powder. The powdered *A. paniculata* leaves (100 g) were soaked in 500 mL of methanol and ethanol solvent and shaken for 72 hours. Afterward, it was filtered and the supernatant was concentrated and evaporated to dryness at 50°C with a rotary evaporator under reduced pressure.

Antioxidant Activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was performed as described by the method given by McCune *et al*., (2002). One mL sample of various concentrations (100–600 µg/mL) of plant extract was added to 3 mL methanolic solution of DPPH solution (0.004%). The mixture was shaken vigorously and incubated at 37°C in the dark for 30 minutes. At the same time, a solution without a sample served as a control. Ascorbic acid was used as the standard and methanol served as control. The absorbance of the sample was measured at 517 nm by using a UV-vis spectrophotometer. The percentage of DPPH scavenging was calculated using the equation as follows:

DPPH radical scavenging activity (%) = {(Abs of control - Abs of sample)/Abs of control} x 100

Where Abs = absorbance

The percentage of DPPH scavenging versus the concentration of samples was plotted. The concentration of the sample is necessary to decrease the DPPH concentration by 50%, denoted as IC_{50} value (µg/mL). The IC_{50} value was calculated by linear regression *(y = a ± bx),* where the extract concentration was as *abscissa* and the percent of antioxidant activity as *ordinate*. All determination was carried out in triplicate. Ascorbic acid was used as a reference compound.

Hydrogen Peroxide (H_2O_2) Scavenging Activity

The ability of plant extract to scavenge hydrogen peroxide (H_2O_2) radical was evaluated by the method of (Ruch *et al*.,1989) with slight modifications. A solution of hydrogen peroxide (40 mM) was prepared in sodium phosphate buffer (pH 7.4). One mL sample of various concentrations (100–600 µg/mL) of plant extract (PE) was added to 2 mL of H_2O_2 buffer solution. The test samples were incubated for 10 minutes at room temperature. The absorbance of hydrogen peroxide was measured at 230 nm against a blank containing the phosphate buffer without H_2O_2, and hydrogen peroxide solution without extract served as control. Ascorbic acid was used as a standard. The hydrogen peroxide scavenging activity percentage was calculated by using the equation as follows:

Hydrogen peroxide scavenging activity (%) = {(Abs of control-Abs of sample)/Abs of control} x 100

Where, Abs = absorbance

The IC_{50} value was calculated by linear regression analysis. Ascorbic acid was used as a reference compound.

Determination of Total Phenolic Compound (TPC)

Total phenolic content (TPC) was measured by the Folin-ciocalteu method, given by McDonald *et al*., (2001). 0.5 mL Folin reagent (Diluted 1:1) was added to 0.5 mL (300 µg mL^{-1}) Plant extract and 2 mL (20%) aqueous sodium carbonate (Na_2CO_3) was added to this reaction mixture and incubated for 15 min at room temperature. Absorbance was recorded at 690 nm. Gallic acid was prepared in methanol and distilled water (1:1) and used as standard. Total phenolic content was expressed in terms of gallic acid equivalent (GAE, mg/g of dry mass), which is a common reference compound.

Phytochemical Analysis

One gram of leaves extract was dissolved in 100 mL ethanol 95%, and the solution was used for preliminary phytochemical screening following the (Harborne,1998) standard methods.

1. ***Phenolic:*** A few drops of $FeCl_3$ (1%) solutions were added to 1-mL plant extract. The formation of green, red, purple, red, or blue-black colour indicated the presence of phenolic.
2. ***Flavonoids:*** 1-mL plant extract solution was mixed with 3 mL of boiled water and incubated for 5 minutes. After that, it was added with 0.05 mg of Mg powder and 1-mL of concentrated HCl then it was shaken. Immediate development of a red, yellow or orange color will indicate the presence of flavonoids.
3. ***Alkaloids:*** Wagner reagent test: Two drops of Wagner reagent were added to 2 mL of extract and mixed well. The appearance of a reddish colour indicates the presence of alkaloids.
4. ***Phytosterol:*** 1-mL of concentrated sulphuric acid was added to the 5 mL extract solution and allowed to stand for 5 minutes. After shaking, the formation of golden yellow color in the lower layer indicates the presence of steroids.
5. ***Tannins:*** 1-mL of plant extract solution was stirred with 5 mL of distilled water. The formation of a blue, blue-black or blue-green colour or precipitation on the addition of (5%) $FeCl_3$ solution indicated the presence of tannins.
6. ***Saponin:*** About 1-mL of plant extract solution was mixed with 3 mL of boiled water and shaken vigorously. If a foam was produced and it was stable for 1 to 2 minutes and persisted on warming, it is evidence of saponin's presence.
7. ***Glycosides:*** 1-mL of plant extract was hydrolyzed with 5 mL hydrochloric acid for a few hours on a water bath and subjected to the Fehling test. 2 mL of extract was added in 2 mL of Fehling solution 1–mL of Fehling's A

solution and 1 mL of Fehling's B solution), mixed well and boiled. The appearance of a yellow or red color precipitate indicates the presence of reducing sugars.

8. ***Amino Acids:*** Added 5% Ninhydrin solution drops in 1-mL of plant extract. The solution was heated in a water bath for 10 minutes, cool and made alkaline. The appearance of an orange precipitate indicates the presence of amino acids.
9. ***Terpenoids:*** Plant extract (5 mL) was mixed with chloroform (2 mL), and concentrated sulphuric acid (3 mL) was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Results

Determination of Antioxidant Activity

The model of scavenging stable DPPH free radicals can be used to evaluate the antioxidative activities relatively quickly. The absorbance decreases as a result of a color change from purple to yellow as the radical is scavenged by antioxidants through a donation of hydrogen to form the stable DPPH-H molecule. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability (Figure 1).

The free radical scavenging activity of *A. paniculata* extract evaluated using the DPPH method is presented in Table 1. Antioxidant activities of extract of plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents and the presence of other constituents in small quantities.

This study determined the free radical scavenging ability of the methanolic and ethanolic extracts. In *A. paniculata*, methanolic extract showed higher scavenging activity (IC_{50}= 394.81 µg/mL) than ethanolic extract (IC_{50}= 401.46 µg/mL) (Table 1). The present study reveals that the antioxidant activity in terms of DPPH scavenging strength was displayed high in methanol extract than in ethanol extract. The high antioxidative capacity of the methanolic extract is due to the higher amount of biologically active substances, such as polyphenols (Manurung & Aryani *et al*., 2019).

Similarly, the hydrogen peroxide (H_2O_2) scavenging activity of the *A. paniculata* plant was observed high in methanolic (IC_{50}= 399.52 µg/mL) followed by the

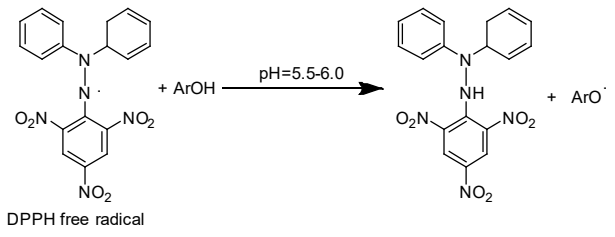

Figure 1: Reduction of DPPH free radical by an antioxidant compound of plant extract.

Table 1: Scavenging effect (%) of *A. paniculata* extract as well as vitamin C by DPPH in different solvents

Sl. no	*Concentration, (µg/mL)*	*% Inhibition of DPPH*		
		Ethanolic	*Methanolic*	*Ascorbic acid*
1.	100	20.01 ± 1.00	24.44 ± 1.00	24.41 ± 0.35
2	200	32.27 ± 0.11	32.34 ± 0.11	37.11 ± 0.11
3	300	46.01 ± 0.89	45.11 ± 0.89	54.02 ± 0.15
4	400	51.41 ± 0.21	54.41 ± 0.21	63.57 ± 0.79
5	500	58.12 ± 1.02	58.72 ± 1.02	85.21 ± 0.15
6.	600	66.19 ± 1.11	83.49 ± 1.11	90.03 ± 0.88
7	IC_{50} (µg/mL)	401.46	394.81	285.69

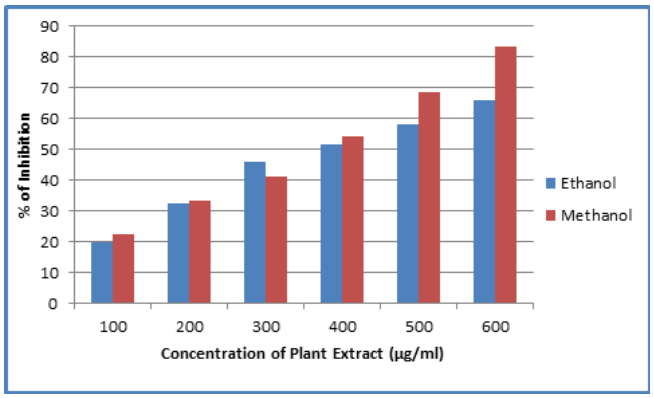

Figure 2: Antioxidant potential of different crude extract of *A. paniculata* against DPPH

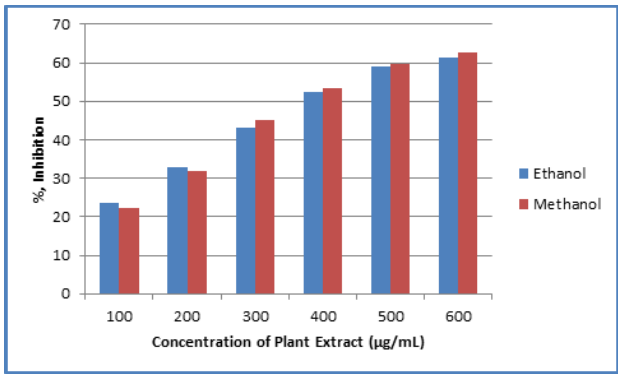

Figure 3: *Antioxidant potential* of different crude extract of A.paniculata against H_2O_2.

ethanolic (IC_{50}= 412.95 µg/mL) extract (Table 2). H_2O_2 scavenging activity relies upon the phenolic content of the plant extract by donating electrons to H_2O_2, thereby neutralizing it into water. In the study of Geetha *et al*., 2013, the scavenging activity of the ethanolic extract of the *Aesculus hippocastanum* was similar to that of ascorbic acid and increased in a dose-dependent manner. H_2O_2 radical scavenging activity of water and ethanol extracts of *C. monogyna* was also reported by Keser *et al*., (2012) and the scavenging activity of different extracts of *E. prostrata* (Sinha and Raghuwanshi, 2016a).

All experiments were carried out in triplicate. Data were expressed as means ± SD. It was found that the *A. paniculata* showed good antioxidant capacities compared

Table 2: Antioxidant activity of of *A. paniculata* extract by H_2O_2 radical Scavenging

Sl. no	*Concentration, (ug/mL)*	*% Inhibition of H_2O_2*		
		Ethanolic Extract	*Methanolic Extract*	*Ascorbic acid*
1.	100	23.54 ± 1.00	22.31 ± 1.00	25.11 ± 0.35
2	200	32.94 ± 0.11	31.77 ± 0.11	35.81 ± 0.11
3	300	43.19 ± 0.89	45.21 ± 0.89	50.02 ± 0.15
4	400	52.41 ± 0.21	53.27 ± 0.21	57.57 ± 0.79
5	500	58.92 ± 1.02	59.72 ± 1.02	65.21 ± 0.15
6.	600	61.29 ± 1.11	62.71 ± 0.11	73.03 ± 0.88
7	IC_{50} (µg/mL)	412.95	399.52	337.55

with vitamin C (Standard antioxidant compound). The results from Table 2 indicate that the radical scavenging activity (% inhibition) of the *A. paniculata* was highest (62.71 ± 1.11) at the concentration of 600 µg/mL. It was noticed that the scavenging activities of the extract were increased with the increase in concentrations. The results indicate that *A. paniculata* is effective in scavenging free radicals and has the potential to be a powerful antioxidant.

Total phenolic content

Phenolic compounds present in plants contain an aromatic ring bearing one or more hydroxyl groups. The GAE method determined the total phenolic content (TPC) in the methanolic leaves extract of *A. paniculata*. The phenolic content was reported as mg/g of GAE in Table 3. In the A.paniculata plant, maximum TPC (296 ± 0.76) was found in methanolic extract, followed by ethanolic (267 ± 1.09). The presence of active metabolites like phenol contents in plant extract depends on the solvent used. Therefore, it was considered that the high antioxidant potential of leaves extract of *A. paniculata* could be attributable to its high amount of phenolic compounds content (Table 3).

Phytochemical analysis

Plants have pharmacological activities attributed to the secondary metabolites which are responsible for essential bioactivities. Phytohemical screening of different extract of leaves of *A. paniculata* revealed the presence of some secondary metabolites as shown in Table 4.

Discussion

Methanol extract revealed the presence of phenol, flavonoids, alkaloids, phytosterols, tannins, saponins, glycosides, amino acids and terpenoids. Ethanol extract showed the presence of phenol, flavonoids, alkaloids, tannins, saponins, amino acids and terpenoids. The phytochemical compounds (alkaloids, phenolic, flavonoids, steroids) detected are known to have medicinal importance. Several environmental factors influence the presence of several secondary metabolites in plants. Phenolic and flavonoid content will be higher in intense drought stress

Table 3: Total phenolic content of *A. paniculata* in two different solvent

Sl.No	*Plant extract*	*Total phenolic content (mg/g GAE)*
1.	Methanol	296 ± 0.76
2.	Ethanol	267 ± 1.09

conditions (Manurung & Kustiawan, *et al* 2019). Soobrattee (2005) reported that phenolic compounds have redox properties and act as an antioxidant. Flavonoids are polyphenolic compounds exhibit several activities such as antioxidant, antibacterial, anti-inflammation, anti-allergy and anti-mutagenic. They are potent antioxidants capable of scavenging ROS due to the presence of a phenolic hydroxyl group (Lahare *et al*., 2020).

Alkaloids are present in several medicinal plants, and it constitutes an appreciable percentage in many available drugs, hence highly essential in disease management. Cardiac glycosides are effective and direct on the cardiac system, supporting the strength of the heart and the rate of contraction when failing (Iwu *et al*., 1983).

The presence of saponins, which are triterpenoid glycosides responsible for the bitter taste and as well-known for their hemolytic effect on red blood cells (Prohp *et al*., 2012). They possess cholesterol-reducing abilities and exhibit structure-dependent bioactivities (Roa *et al*., 1995). The saponins content of plants also helps in fighting pathogens and boosting the immune system.

The presence of terpenoids indicates that steroidal compounds could be present, which are of great use/ importance in synthesizing sex hormones synthetic compounds (Okwu *et al*.,2001).. Thus, potentially making *A. paniculata* leaves a great medicinal herb for large varieties of diseases (Trease *et al*.2002).

A. paniculata Nees is a valuable medicinal herb with many important bioactive compounds such as diterpenoids, flavonoids, and polyphenols. Numerous human ailments are treated and prevented by it. The *A. paniculata* leaves were collected from a local garden and extracted in methanol and ethanol solvent. The free radical scavenging activity of *A. paniculata* extract against 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and & Hydrogen peroxide (H2O2) radical was determined. Methanolic extract showed higher scavenging activity than ethanolic extract. The IC_{50} value of methanolic and ethanolic extract of the plant in DPPH was 394.81 and 401.46 µg/mL, while in vitamin C was 285.69 µg/mL. However, the IC_{50} value of the Hydrogen peroxide (H_2O_2) radical for methanolic extract was higher 399.52 µg/mL than for ethanolic extract 412.95 µg/mL as compared with vitamin C as a reference antioxidant compound, 337.55 µg/mL.Thus, methanolic extracts were most potent against 50% inhibition, IC_{50} value showed a good correlation with total phenolics content. Therefore, *A. paniculata* has strong antioxidant potential. The present study exhibited

Table 4: Phytochemical screening of plant in different solvents

Sl. No	*Phytochemicals*	*Test performed*	*Observation*	*Methanol Extract*	*Ethanol Extract*
1	Phenols	Ferric chloride test	green, red, purple, red, or blue-black	±	±
2	Flavonoids	Alkaline reagent	red, yellow or orange coloration formation	±	±
3	Alkaloids	Wagner's test	Reddish color	±	±
4	Phytosterol	Salkowski test	Golden yellow color	±	-
5	Tannins	Ferric chloride test	blue, blue-black or blue-green color	±	±
6	Saponins	Frothing test	Foam formation	±	±
7	Glycosides	Fehling test	Yellow or red color precipitate	±	-
8	Amino acids	Ninhydrin test	Orange precipitate	±	±
9	Terpenoids	Chloroform test	Brown ring formation	±	±

Note : (±) means Presence,(-) means Absence of Phytochemicals

the presence and absence of phyto-compounds in each solvent extract. It was found that a maximum number of phytochemicals in methanol extract. Therefore its effectiveness as a medicinal plant is due to the presence of various phenolics and antioxidant compounds, individually or synergistically.

Conclusions

The present study has been concerned with determining the chemical composition and antioxidant activity of the leaves extract of *A. paniculata*. The antioxidant activity was determined by DPPH and H_2O_2 free radicals. The antioxidant property and total phenolic content was significantly varied in two different solvents. The methanol extract showed higher antioxidant activity than the ethanol extract. It was also found that methanol extract showed the maximum number of phytochemicals than ethanol extract. *A. paniculata* leaves extract showed good antioxidant capacities compared with vitamin C (Standard antioxidant compound). Due to its various therapeutic applications, it is widely cultivated in many parts of the world, and its relevance as a medicinal plant is constantly increasing.

Acknowledgements

The author acknowledges her indebtedness and thanks to the Principal, DBS PG College, Dehradun for the moral support and providing all necessary facilities.

Conflicts of Interest

Nil

References

Alok, S., Jain, S. K., Verma, A., Kumar, M., Mahor, A., & Sabharwal, M. (2014). Herbal antioxidant in clinical practice: A review. Asian Pacific Journal of Tropical Biomedicine, 4(1), 78–84. https://doi.org/10.1016/S2221-1691(14)60213-6

Bhatnagar, A. (2023). A comprehensive review of kalmegh's biological activities (Andrographis paniculata). International Journal of Pharmacy and Pharmaceutical Sciences, 15(2) 1-7, doi:10.22159/ijpps.2023v15i2.46705.

Bhatnagar, A.(2023) Chemical constitutents of Andrographis paniculata (Burm.F)Nees :A review". International Journal of Pharmaceutical Sciences and Research. 14(7)1000-1008, doi:10.13040/IJPSR.0975-8232.14(7).1000-10008.(accepted)

Chakravarti, R.N.; Chakravarti, D (1951). Andrographolide, the Active Constituent of Andrographis paniculata Nees. A Preliminary Communication. Ind. Med. Gaz. 86, 96–97.

Chander R., Srivastava V, Tandon J S, and Kapoor N. K.(1995). Antihepatotoxic activity of diterpenes of Andrographis paniculata (Kal-Megh) against Plasmodium berghei-induced hepatic damage in Mastomys natalensis. Int. J Pharmacogn.33(2):135–138

Geetha, RV., Roy, A., Sitalakshmi, T. (2013) In Vitro Antioxidant and free Radical Scavenging activity of the Ethanolic extract of Aesculus hippocastanum. International Journal of Drug Development and Research, 5(3): 403-407.

Handa S.S. and Sharma A.(1990). Hepatoprotective activity of andrographolide from Andrographis paniculata against carbon tetrachloride. The Indian Journal of Medical Research. 92: 276–283.

Harborne, J.B.(1998) Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis‖, 3rd ed. New York: Chapman and Hall Int Ed, 1998:234-245.

Iwu MM(1983) Hypoglycemic properties of Bridelia furruginear leaves. Fitoterapia 1983; 54: 243 -248.

Joshi R.K,, Satya I.P., and Setzer W.N.(2016). Himalayan aromatic medicinal plants: a review
of their ethnopharmacology, volatile phytochemistry and biological activities. Medicines; 3(1):1-55.

Kalaiselvan, A., Gokulakrishnan, K., & Anand, T. (2012). Gas chromatography-Mass Spectrum analysis of bioactive components of the ethanol extract of *Andrographis paniculata*. Journal of Pharmaceutical and Biomedical Sciences , 20(20), 13–16.

Keser, Serhat & Celik, Sait & Türkoğlu, Semra & Yilmaz, Okkes & Turkoglu, Ismail. (2012). Hydrogen Peroxide Radical Scavenging and Total Antioxidant Activity of Hawthorn. Chem J. 2.

Khajuria A.K., Manhas R.K., Kumar H, Bisht N.S.(2021). Ethnobotanical study of traditionally used medicinal plants of Pauri district of Uttarakhand, India. J Ethnopharmacol,Aug 10; 276: 114204. doi: 10.1016/j.jep.2021.114204. Epub 2021 May 15. PMID: 34000367.

Lahare, R. P., Dashahre, A. K., Bisen, Y. K., & Yadav, H. S. (2020). Phytochemical Analysis of Andrographis paniculata and Catharanthus Rosea from Korba District , Chhattisgarh ,.

9515, 423–431. https://doi.org/10.36347/sajb.2020.v08i12.003

Lee S, Morita H, Tezuka Y (2015). Preferentially cytotoxic constituents of Andrographis paniculata and their preferential cytotoxicity against human pancreatic cancer cell lines. Nat. Prod. Commun.; 10 (7): 1153–1158

Liu J, Wang Z.T, and Ge B. X. (2008). Andrograpanin, isolated from Andrographis paniculata, exhibits anti-inflammatory property in lipopolysaccharide-induced macrophage cells through down-regulating the p38 MAPKs signaling pathways. International Immunopharmaology. ; 8(7): 951–958.

Manurung, H., Aryani, R., Nugroho, R. A., Sari, Y. P., & Chernovita, R. (2019). Phytochemical Analysis And Antioxidant Activity Of Leaves Extracts Of Endemic Plant Jahe Balikpapan (Etlingera Balikpapanensis A . D . September.

Manurung, H. Kustiawan,W Kusuma, I.W. Marjenah, Nugroho, R.A.(2019). Growth, phytochemical pofile, and antioxidant activity of cultivated tabat barito (Ficus deltoidea Jack.) under drought stress‖ International Journal of Biosciences, 14(1): 366-378.

McCune, L. M., & Johns, T. (2002). Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the Indigenous Peoples of the North American boreal forest. Journal of Ethnopharmacology, 82(2–3), 197–205. https://doi.org/10.1016/S0378-8741(02)00180-0

McDonald,S., Prenzler, PD, Autolovich, M., Robards, K.(2001) Phenolic content and antioxidant activity of olive extract. Food chemistry, 73(1) :73-84.

Okwu DE (2001. Evaluation of the chemical composition of indigenous spices and flavouring Agents. Global J. Pure Appl. Sci 7(3): 455-9.

Prohp TP, Onoagbe, I.O (2012). Determination of phytochemical composition of the stem bark of triplochiton scleroxylon k. schum. (sterculiaceae). International Journal of Applied Biology and Pharmaceutical Technology 2012; 3 (2): 68-76.

Qin, L.H, Kong, L, Sh,i G.J, Wang, Z.T, Ge, B.X(2006) Andrographolide inhibits the production of TNF-‖ and interleukin 12 in lipopolysaccharide-stimulated macrophages: role of mitogen-activated protein kinases. Biological and Pharmaceutical Bulletin.29(2): 220–224.

Roa R.R, Babu R.M, Rao M.R.V. (1995). Saponins as anticarcinogens. The J. Nutr; 125: 717-24

Ruch, R.J., Cheng, S.J., and Klaunig, J.E. (1989) Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 10,1003-1008

Saxena S, Jain D.C, Bhakuni R.S, Sharma R.P.(1998).Chemistry and pharmacology of Andrographis species. Indian Drugs; 35: 458-67.

Sinha, S. and Raghuwanshi, R. (2016a) Phytochemical screening and antioxidant potential of Eclipta prostrata (L) L-a valuable herb. International Journal of Pharmacy and Pharmaceutical Sciences, 8(3), pp.255-260.

Soobrattee, M.A. Neergheen, V.S. Luximon-Ramma, A. Aruoma, O.I. Bahorun, O.T.(2005). Phenolics as potential antioxidant therapeutic agents: mechanism and actions‖, Mutat. Res. Fundam. Mol. 579: 200–213. Lee S, Morita H, Tezuka Y (2015). Preferentially cytotoxic constituents of Andrographis paniculata and their preferential cytotoxicity against human pancreatic cancer cell lines. Nat. Prod. Commun. 10 (7): 1153–1158.

Trease G.E, Evans W.C (2002). Phytochemicals. In: Pharmacognosy. 15th ed. Saunders Publishers, London; p. 42- 4, 221-9, 246-9, 304-6,331-2, 391-3.