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Extraction of polar and non polar flavonoids from Azadirachta indica leaves and study their Antioxidant activity

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Abstraction
Azadirachta Indica common name Neem. *Azadirachta Indica* is one of the most versatile medicinal plants having a wide spectrum of biological activity due to the presence of large number of bioactive compounds. The present study was conducted to evaluate polar and non polar flavonoids from *Azadirachta indica* leaves by using column chromatography technique. And studies their antioxidant activity by taking 0.02% of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay method.

Key words :- Polar & Non Polar flavonoids, Antioxidant activity, DPPH, Azadirachta Indica leaves

Introduction

Azadirachta Indica (Neem) tree belongs to the Meliaceae family and it origin from India , Assam and Burma of South Asia. The neem tree has been used traditionally for centuries in both agriculture and medicine it has been used in Ayurvedic medicine for over 4000 years. Throughout the world refer to the neem tree as a village pharmacy because it cures diseases and disorders ranging from bad teeth and bed bugs to ulcers and malaria. Apart from the medicinal benefits, the insecticidal properties of the leaves, bark and oil which can be effective as pesticides, herbicides, fungicides and weedicides in agriculture.
Neem is known to have the potential to produce strong antioxidant activity it is known that antioxidants can counteract free radicals due to an oxidation reaction. Free radicals are molecules that do not have a partner so that the molecule becomes unstable and has high reactivity. As the number continues to increase, the amount of antioxidants and free radicals becomes unbalanced, which can cause oxidative cell stress. Neem produce secondary metabolites such as phenolic and flavonoids which is responsible for antioxidant activity. Many biologically active compounds can be extracted from neem, including terpenoids, phenolic compounds, carotenoids, steroids, and ketones. Other pharmacological actions also be shown such as antipyretic, antifungal, antibacterial, anti-inflammatory, antiviral, antimalarial etc.

Material and Methods

1.Preparation of Neem leaf extract

Grind neem leaves into a fine powder and extract them using a suitable solvent like ethanol or methanol. This step will extract both polar and non-polar compounds.

2.Detection of flavonoid*s*

Neem leaves extract was taken and few drops of 10% lead acetate solution was added. Appearance of yellow colour precipitate indicates the presence of flavonoids

3.Extraction of polar & non polar flavonoids by using column chromatography

Prepare a column filled with a stationary phase (e.g., silica gel) and pack it tightly.Apply the sample to the top of the column and let it absorb onto the stationary phase. Start eluting the compounds by passing a series of solvent mixtures through the column.
Initially, use a non-polar solvent like hexane or dichloromethane to elute non-polar flavonoids. Then gradually increase the polarity of the eluent by adding a more polar solvent like ethyl acetate or methanol to elute polar flavonoids. Collect the eluted fractions in test tubes or flasks. Each fraction represents a mixture of compounds with similar polarity. Analyse each fraction using thin-layer chromatography (TLC) to identify the presence of flavonoids. Compare the retention times or Rf values with standard compounds.
Rf value =Distance travelled by solute / Distance travelled by solvent.

4.Study of Antioxidant Activity By DPPH

The antioxidant activity of the ethanol extracts of *Azadirachta indica* leaves were assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The diluted working solutions of the test plant extracts were prepared in ethanol. 0.004% of DPPH was prepared in ethyl alcohol and 3 ml of this solution was mixed with 3 ml of sample solutions. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV Visible spectrophotometer. Alcohol (3 ml) with DPPH solution (0.004%, 3 ml) was used as blank.
The optical density was recorded and % inhibition was calculated using the formula given below:
Percentage (%) Inhibition of DPPH (% AA) = A – B × 100 ÷ A
Where A=Optical density of the blank

B=Optical density of the sample.

Result and Discussion

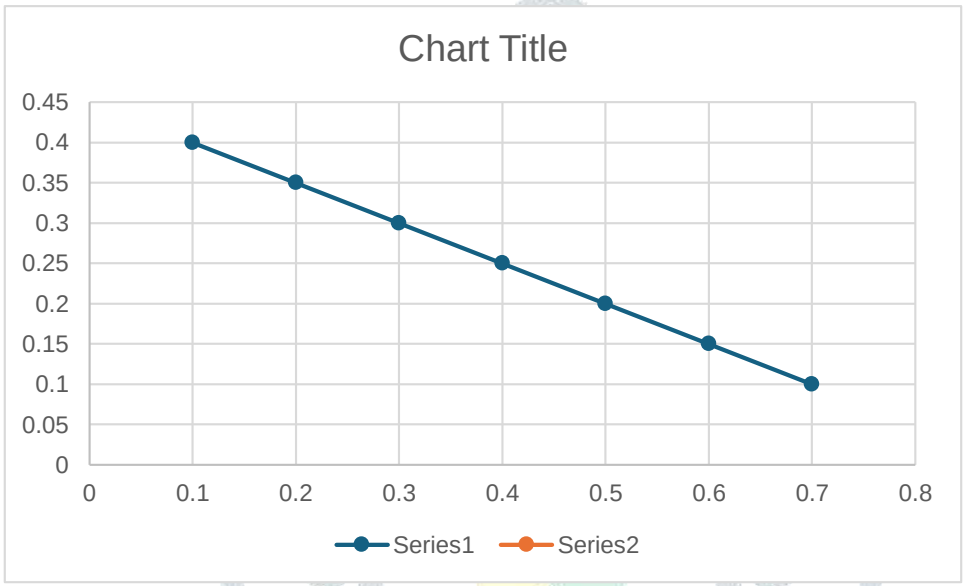
Polar and non polar flavonoids found in neem leaves and it analyse by using TLC method. TLC plat showing spots having different RF values that are 0.5, 0.4, 0.7, 0.3 etc. And study antioxidant activity by using DPPH solution and different contractions of sample.

The stock solution 1ml of ethanol was prepared. The required dilutions 0.1ml to 0.9 ml were prepared by appropriate dilutions. The optical density and percent antioxidant activity were calculated.

Optical Density And Percent Antioxidant Activity For Ethanolic Extract of Azadirachta indica leaves (O.D. of Black DPPH = 0.47)

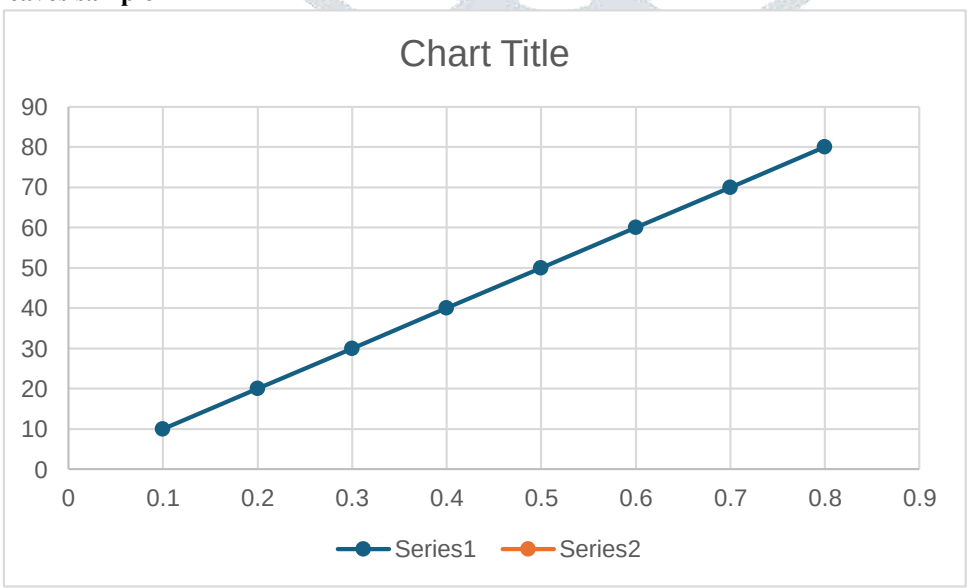
Con. mg/ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7
OD of sample	0.38	0.34	0.29	0.25	0.22	0.18	0.15
% AA	19%	27%	38%	46%	53%	61%	68%

Decrease in O.D sample with increase in conc. Neem leaves sample



IC_{50} = 0.265

Increase in percent antioxidant activity with increase in con. of neem leaves sample



IC_{50}= 43.5

Conclusion

Form about study conclude that both polar and non polar flavonoids present in Azadirachta indica leaves. It is confirmed by Lead Acetate Test and antioxidant activity also be study by using graphical representation that is decrease of O.D. value and increase in %AA with increase in concentration of neem leaves extract.

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References

1.Babu.S, Naik.K, Ramanjaneyulu.K, Antioxidant activity of stem bark of Azadirachta indica in different scavenger activity IJPCBS 2016, 6(3), 280-286 ISSN: 2249-9504

2.Seriana.I, Akmal M, Wahyuni.S , Khairan.K And Sugito. Phytochemical characterization of neem tree leaves Ethanolic extraction RASAYAN J. Chem. Vol. 14 No. 1 343-350 (2021).

3.Brahmachari G. Neem an omnipotent plant: A retrospection.chemBiochem (2004) 5. 408-421.

4.Schmuterer.H , Properties and potential of natural pesticides from the neem tree. Annu. Rev. Entomol. (1990). 35:271-97

5. Asif. M. Antimicrobial Potential of Azadirachta indica Against Pathogenic Bacteria and Fungi.Journal of Pharmacognosy and Phytochemistry. Vol. 1 No. 4 (2012)

6. Adyanthaya.S, Pai.V, Antimicrobial potential of the extracts of thebTwigs of Azadirachta indica. Journal of Medicinal Plants Studies 2014; 2(6): 53-57

7.Shareef.M , Akhtar.S, Neem and it's Potential for safeguarding health prevention and treatment of disease. Matrix Science Medica (MSM) 2(1) (2018) 04-08.

8.Martínez. A, Munguía.A and Inungaray.M. Evaluation of Antioxidant property of Neem. National Congress of biotechnology and bioengineering.(2010).

9.Ghimeray.A, Jin.C, Ghimire.B, Antioxidant activity and quantitative estimation of Azadirachtin and nimbin in Azadirachta Indica A. Juss Grown in foothills of Nepal African Journal of Biotechnology Vol. 8 (13), pp. 3084-3091, 6 July, (2009)

10.Al-Jadidi. S. Hoda, Hossain.A, Studies on total phenolics, total flavonoids and Antimicrobial activity from the leaves crude Extracts of neem traditionally used for the Treatment of cough and nausea beni-suef university journalod besic and applied science xxx ,(2015).I-6.

11.Nahla M. Abd El-Aziz. Insecticidal activity of Azadirachta indica (Sapindales, Meliaceae) extracts against Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) Current Science International.Vol. 06 Issu:04 (2017) Pages: 891-899

12.Hismath. I. Hismath, I., Wan Aida, W. M. And Ho, C.W.Optimization of extraction conditions for phenolic compounds From neem. International Food Research Journal 18(3): 931-939.