Research Article

In vitro pharmacological investigation of fruit of Acacia nilotica

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ABSTRACT

The famous medium-sized Acacia nilotica tree, often known locally as "Babul" or "Kikar," is widely distributed throughout tropical and subtropical regions. It has a wide variety of possible medical applications and antioxidant action. This study's goal was to ascertain the fruits of Acacia nilotica's Invitro anti-inflammatory and antioxidant activities. Acacia nilotica hydroalcoholic extract's Invitro anti-inflammatory and anti-oxidant activities were assessed. The significant activity of Inhibition of Protein denaturation shown a maximum of 76% inhibition at 500 µg/ml concentration. From the hydroalcoholic extract 400 g/ml showed a maximum of 99.85% of DPPH radical scavenging activity.

Key words: Protein denaturation, Acacia nilotica, DPPH, free radical scavenging

India's variety of medicinal plants is its true source of wealth. Since the beginning of time, numerous varieties of medicinal herbs and their preparation have been used. Developing nations have a diverse range of medicinal plants that could be used to create novel biologically active compounds. Drug development from plants must inevitably entail a multidisciplinary strategy in terms of a contemporary research project. Mineral, plant, and animal products were the primary sources of medicines for a very long time [1].

There is evidence that almost all ancient civilizations used herbs for the treatment of illnesses and for reviving bodily systems. Herbal medicines are typically made from a single extract, a fraction of that extract, or a combination of fractions and extracts from various plants. These substances must be carefully standardized for safety, efficacy, and cost-effectiveness [2–4]. Along with other areas of human activity, the study of medicine and related sciences has advanced quickly. The body's antioxidant defence mechanisms are only effective when the number of free radicals is within the normal physiological range; otherwise, oxidative stress, which can lead to tissue damage and consequent illnesses [5, 6].

MATERIAL AND METHODS

Method of Extraction

Weighed 45 gm of powdered drug of fruits of Acacia nilotica and it was extracted in soxhlet apparatus by using hydroalcoholic (viz. water: ethanol) solvent. Soxhlet apparatus filled with powder of Acacia nilotica fruit then added the solvent system into it and started heating at 55 0 C after completing one cycle of the assembly. Stopped heating when solvent became colourless then extract was collected and air dried.

Invitro Anti-Inflammatory Activity

Inflammation is the body's natural defence mechanism and is characterized by pain, swelling, heat generation, and loss of function in the wounded area. The location and severity of the damage will determine how much function the afflicted part loses. The body's reaction to an accidental cut is comparable to the reaction to other types of tissue injury brought on by burns from heat, radiation, bacterial, or viral attack since inflammation is the body's generalised defence mechanism. Inflammation is a complicated process that is linked to pain and includes things like increased protein denaturation, increased vascular penetrability, and layer change. Prostaglandin, kinins, and histamine are chemical mediators released by injured bodily tissue (cells) during inflammation. Vasodilation and capillary permeability are increased as a result. This causes the blood flow to the injured location to increase. Various in vitro and in vivo models are employed to research anti-inflammatory efficacy. Acacia nilotica invitro model is assessed for the current study of anti-inflammatory activities.

Invitro Protein Denaturation Inhibition Bioassay

Chemicals used for the activity: BSA (Bovine Serum Albumin), NaCl, Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Methanol, Standard diclofenac sodium.

Preparation of reagent % Bovine serum Albumin: Dissolve 5 gm. of BSA in phosphate buffer saline of pH 6.3

Phosphate buffer saline of pH 6.3: Adjust the pH of the solution to 6.3 by adding HCL after dissolving 0.68 grammes of potassium dihydrogen phosphate, 0.895 grammes of disodium hydrogen phosphate, and 3.50 grammes of sodium chloride.

Preparation of standard solution: The standard stock solution of diclofenac sodium in methanol was generated at a concentration of 10,000 mg/ml, and three different concentrations of 100, 200, and 500 mg/ml were prepared from this stock solution.

Test solution preparation: Using methanol as the solvent, stock solutions of various fruit extracts containing 10,000 g/ml were created. Three distinct concentrations of 100, 200, and 500 g/ml were made from this stock solution.

Procedure: The procedure for the current study's assay of protein denaturation inhibition is provided below. In this assay, 0.1 ml of test solution of Acacia nilotica fruit extract of different concentrations (100 g/ml, 200 g/ml, and 500 g/ml produced in methanol) and 0.9 ml of bovine serum albumin (5 percent aqueous solution) were taken in different test tubes. In place of the test solution for the control, 0.1 ml of distilled water was employed. For 0.1 ml of diclofenac as is customary. Instead of the test solution, sodium was used (different concentrations of methanol were made, namely 100, 200, and 500 g/ml). The mixture was incubated at 37 °C for 5 min, then test tubes were heated at 55 °C for 3 min, and finally allowed to cool. Each test tube received 2.5 ml of phosphate buffer saline with a pH of 6.3 after being cooled. At 660 nm, the absorbance was spectrophotometrically quantified [14].

Standardization: By employing standardised diclofenac sodium, the Invitro serum albumin denaturation inhibition bioassay was standardised.

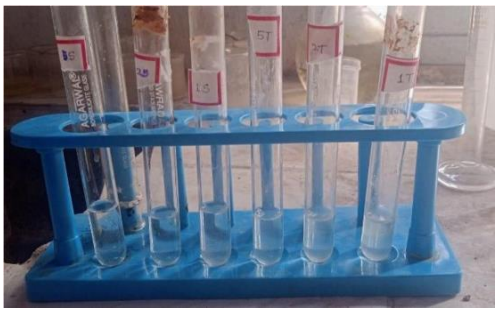


Fig. no. 1: Images Of Anti – inflammatory Activity

Calculation: The inhibition to the protein denaturation was measured as

$$\% \text{ Inhibition} = \frac{\text{Abs of control} - \text{Abs of treated}}{\text{Abs of control}} \times 100$$

Invitro Anti-Oxidant Activity

Oxidation: The chemical reaction known as oxidation involves the transfer of an electron or hydrogen atom from a material to an oxidising agent. Free radicals are produced as a result of the oxidation reaction.

Antioxidants: By preventing the start of oxidative chain reactions, antioxidants are substances that prevent or delay the oxidation of other molecules. The reducing agents are frequently antioxidants.

DPPH Radical Scavenging Activity

Principle: Due to its unpaired electron, the persistent free radical known as DPPH (1, 1-diphenyl-2-picrylhydrazyl) has a rich purple color. When an antioxidant provides the DPPH with an electron, the DPPH's deep violet color decolorizes to a pale yellow non-radical form. When a solution of DPPH is mixed with a solution of a substrate that can give off a hydrogen atom, at that point this gives rise to the reduced form with the loss of this violet shading. This change in colour of DPPH and the subsequent drop in absorbance are observed spectrophotometric ally at a maximum of 517nm.

Chemicals used for the activity: DPPH (2, 2-diphenyl-1-picrylhydrazyl), Ascorbic acid, Methanol.

Preparation of reagents:

1. DPPH solution: Dissolve 0.025 mg of DPPH in 1000 ml of methanol. Prepare fresh solution while using.
2. Standard solution (Ascorbic acid solution): Dissolve 100 mg of ascorbic acid in 100 ml of methanol.
3. Test solution (Extract solution): dissolve 50 mg of test sample in 50 ml of methanol.

Procedure: Using a methanolic solution of DPPH, the ability of Acacia nilotica fruit extracts to scavenge free radicals was evaluated. A free radical is DPPH. Antioxidants convert DPPH to the 517 nm-measured 2, 2diphenyl-1-picrylhydrazine. The common antioxidant utilised is ascorbic acid. The assay is carried out utilising the brand Williams et al. method. The reaction combination used to assess this activity contains 3.9 ml of methanolic DPPH solution and 0.1 ml of various extract solutions (containing 25, 50, 100, 150, 200, and 400 g/ml). Instead of extract, 0.1 ml of ascorbic acid was utilised for the standard. Moreover, 0.1 ml of methanol was employed for the blank preparation. This combination was incubated at room temperature for 30 minutes in the dark. 517 nm absorbance measurement. All extracts' percentage DPPH radical scavenging activity was evaluated and compared to the reference value [14].

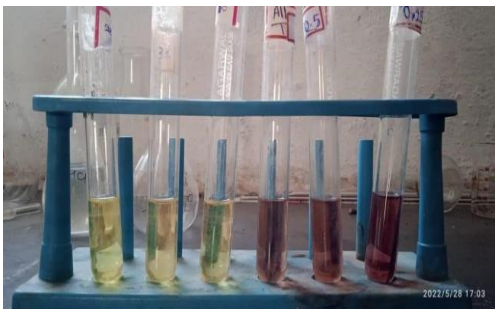


Fig. no. 2: Images of Antioxidant Activity

Standardization: Ascorbic acid was used to standardise the Invitro antioxidant DPPH free radical scavenging activity.

Calculation: The percent inhibition was measured as

$$\% \text{ Inhibition} = \frac{\text{Abs of control} - \text{Abs of treated}}{\text{Abs of control}} \times 100$$

RESULT

Invitro Anti-inflammatory Activity:

Invitro Protein Denaturation inhibition bioassay: Bioassay for In Vitro Suppression of Protein Denaturation In vitro percentage (percent) inhibition of protein denaturation caused by heat of standard Diclofenac sodium in table no.1

Invitro Antioxidant Activity: Invitro Antioxidant Activity: DPPH free radical scavenging activity

Table No. 1: Heat induced protein Denaturation inhibition of standard Diclofenac sodium

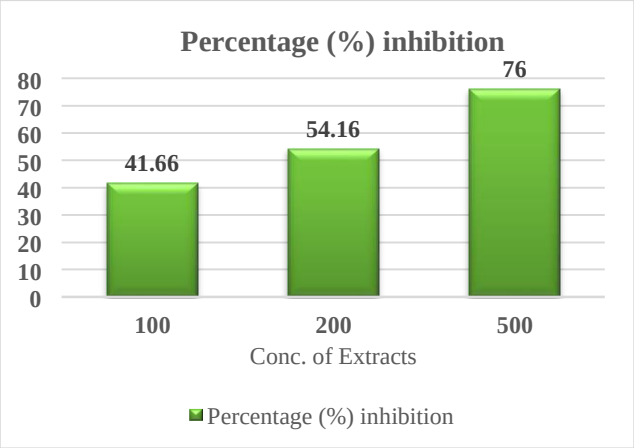
Standard Drug	Concentration in µg/ml	Absorbance	(%) inhibition
Diclofenac sodium	100	0.115	38.50
	200	0.076	59.35
	500	0.043	77.00

Table No. 2: Heat induced protein denaturation inhibition of different extracts of *Acacia nilotica* Fruits.

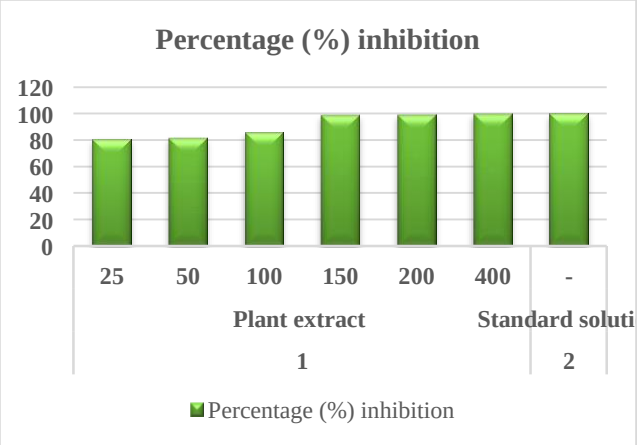
Plant Extract	Concentration in µg/ml	Absorbance	(%) inhibitin
Control	-	0.024	-
Hydroalc oholic extract	100	0.014	41.66
	200	0.011	54.16
	500	0.006	76

Table No. 3: Percentage of DPPH free radical scavenging activity of fruit extract of *Acacia nilotica.*

Solution	Con. in µg/ml	(%) inhibition
Plant extract	25	80.6
	50	81.67
	100	85.67
	150	99.21
	200	99.35
	400	99.85
Standard solution	-	99.87



Graph No. 1: Percentage inhibition of protein denaturation at different concentration of Fruit extract of *Acacia nilotica*



Graph No. 2: DPPH radical scavenging activity of Extract of *Acacia nilotica* fruits

DISCUSSION

Acacia nilotica contain number of phytochemicals such as Alkaloids, Flavonoids, Phenols and terpenes which are responsible for Anti-inflammatory and antioxidant activity. Inflammation include events such as expansion of vascular permeability and increment of protein denaturation. Inflammation leads to tissue injury which causes release of chemical mediator such as prostaglandins, kinins and histamine. These mediators leads to vasodilation which causes increase blood flow at the site of injury. Antiinflammatory compounds gives inhibitory effect against inflammation by inhibiting release of chemical mediators

The protein denaturation inhibition assay is being used for the first time in this work to evaluate the antiinflammatory

efficacy of the fruit extracts of the ethnomedical herb Acacia nilotica. Heat causes denaturation of proteins. Evaluation of hydro-alcoholic extract's inhibition of protein denaturation. According to the results of the current investigation, percentage inhibition rises as concentration goes from 100 g/ml to 500 g/ml. In comparison to the other fruit extracts of Acacia nilotica, these all exhibit the highest percentage inhibition of 76 percent at 500 g/ml concentration as shown in table no. 2. Following that, the % inhibition of standard Diclofenac sodium is compared to that of the hydro-alcoholic extract. At a concentration of 500 g/ml, diclofenac sodium exhibits a 77 percent inhibition in percentage as shown in table no. 1. ROS include free radicals which is produced by oxidation process. But the excessive generation of free radicals leads to oxidative stress and causes various diseases. Antioxidant compound reduces the generation of free radicals and prevent the disease caused by oxidative stress. The Phenolics and Flavonoids leads to prevention of ROS formation by inhibiting oxidative enzyme.

The DPPH free radical scavenging assay is being used for the first time in this work to evaluate the anti-oxidant activity of the fruit extract of the ethnomedicinal herb Acacia nilotica. The hydro-alcoholic and aqueous concentration's activity to scavenge DPPH free radicals has come to an end. In comparison to the other fruit concentrates of Acacia nilotica, the 400 g/ml concentration of the Acacia nilotica fruit extract exhibits considerable DPPH free radical scavenging activity of 99.85 percent as shown in table no. 3. The common antioxidant utilised is ascorbic acid nociceptive action it blocks the pain sensation caused by inflammation.

Protective extent of Acacia nilotica: Acacia nilotca provide effect against peripheral as well as CNS inflammation. It shows antinociceptive action that it blocks the pain sensation caused by inflammation. It act as antiinflammatory by inhibiting formation of pain mediators i.e. prostaglandins and kinins at peripheral target sites. Acacia nilotica shows the CNS depressant activity which helps to treat pain attacks, anxiety. It increases the activity of GABA receptor which shows calming effect which used to treat anxiety, pain sensation and insomnia.

CONCLUSION

In the current investigation, the hydroalcoholic extract's protein denaturation inhibition assay method is used to assess and confirm the invitro anti-inflammatory effect. These analyses lead to the conclusion that, when compared to the industry standard, diclofenac sodium, the hydroalcoholic extract significantly inhibits protein denaturation by 76% at a concentration of 500 g/ml. DPPH radical scavenging activity is another method of confirming the in vitro antioxidant activity of Acacia nilotica fruit. According to the results of this test, the hydroalcoholic extract exhibits 99.85 percent radical scavenging activity at a concentration of 400 g/ml.

For this test, the standard antioxidant is ascorbic acid. From all of the research, it has been determined that the fruit extract of Acacia nilotica may have antioxidant and anti-inflammatory properties.

REFERENCES

1. De Pasquale C, Pistorio ML, Veroux P, et al. Quality of life and mental health in kidney transplant recipients during the COVID-19 pandemic. Front Psychiatry. 2021, 12: 10.3389/fpsyt.2021.645549
2. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. IJP. 2000, 32.
3. Patwardhan B. Ayurveda: The 'Designer' medicine: A review of ethnopharmacology and bioprospecting research. Indian Drugs. 2000, 37.
4. Kelly GS. Nutritional and botanical interventions to assist with the adaptation to stress. Altern. Med. Rev. 1999, 4.
5. Sahoo N, Manchikanti P, Dey S: Herbal drugs: Standards and regulation. Fitoterapia. 2010, 81: 10.1016/j.fitote.2010.02.001
6. Cheeseman KH, Slater TF: An introduction to free radical biochemistry. Br Med Bull. 1993, 49: 10.1093/oxfordjournals.bmb.a072625
7. Ajith TA, Janardhanan KK. Indian medicinal mushrooms as a source of antioxidant and antitumor agents. J. Clin. Biochem. Nutr. 2007, 40: 10.3164/jcbn.40.157
8. Sies H. Oxidative stress: Oxidants and antioxidants. Exp. Physiol. 1997, 82: 10.1113/expphysiol.1997.sp004024
9. Zhang L, Ravipati AS, Koyyalamudi SR, et al. Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds. J Agric Food Chem. 2011, 59: 10.1021/jf203146e
10. Tang SY, Whiteman M, Peng ZF, et al. Characterization of antioxidant and antiglycation properties and isolation of active ingredients from traditional chinese medicines. Free Radic Biol Med. 2004, 36: 10.1016/j.freeradbiomed.2004.03.017
11. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004, 74: 10.1016/j.lfs.2003.09.047
12. Dragland S, Senoo H, Wake K, et al. Several culinary and medicinal herbs are important sources of dietary antioxidants. J Nutr. 2003, 133: 10.1093/jn/133.5.1286
13. Hendra R, Ahmad S, Oskoueian E, et al. Antioxidant, Anti-inflammatory and Cytotoxicity of Phaleria macrocarpa (Boerl.) Scheff Fruit. BMC Complement Altern Med. 2011, 11: 10.1186/1472-688211-110
14. Dafallah AA, Al-Mustafa Z. Investigation of the antiinflammatory activity of acacia nilotica and hibiscus sabdariffa. Am J Chin Med. 1996, 24: 10.1142/s0192415x96000323

How to cite this article: Pradnya Shinde, Shraddha Kadav, Ashwini Kamble, Ashpak Tamboli. *In vitro* pharmacological investigation of fruit of Acacia nilotica. Indian J Pharm Drug Studies. 2023; 2(1) 33-36.

Funding: None *Conflict of Interest: None Stated*