

INVITRO EVALUATION OF ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITY BY USING ETHANOL EXTRACT OF BANANA PEEL

****T.VARALAKSHMI¹, M.HEMALATHA², K. SRIDEVI³, DR.S. ASHOK KRISHNAN⁴, DR.G. ALAGU MANIVASAGAM⁵**

^{1,2,3,4} Department of pharmacology, school of pharmaceutical sciences & technologies, Jawaharlal Nehru Technological University, Kakinada, Andhra Pradesh-533 003, India.

ABSTRACT:

The present study of the investigation was carrying out by evaluation of the in vitro antioxidant and anti-inflammatory activity of Banana peel. The anti-inflammatory activity was evaluated by the Protein denaturation method. Radial scavenging activity and Hydrogen peroxide scavenging activity assay was used to evaluate the antioxidant potential. Phytochemical tests of ethanol extract of Banana peel showed the presence of flavonoids, alkaloids, steroids, glycosides, saponins, phenols, tannins, carbohydrates and proteins. Total flavonoid and phenol content of ethanol extract of banana peel were found to be 138.7 & 149.9 µg/ml respectively. In the case of anti-inflammatory activity, the maximum percentage inhibition was observed as 78.22% at 500 µg/ml concentration. The maximum percentage inhibition by radial scavenging activity assay and hydrogen peroxide scavenger assay was observed as 94.15% and 92.13% respectively at 500 µg/ml concentration for antioxidant activity. Present results highlight the role of ethanol extract of Banana peel for its anti-oxidant and anti-inflammatory activity.

Key words: *Banana peel, phytochemical analysis, anti-oxidant, anti-inflammatory, Protein denaturation method, DPPH Assay, hydrogen peroxide scavenger activity.*

INTRODUCTION

Antioxidants are capable of inhibiting other molecules which belong to classes of a chemical molecule. Free radicals are formed by an oxidation reaction. These free radicals damage cells by starting chain reactions and highly unstable. A large collection of scientific evidence recommends that chronic oxidative stress increases the progression of panic conditions such as cancer, diabetes, inflammation and heart disease [1]. These applies to signal transduction, gene transcription and regulation of cell-soluble guanylate cyclase activity, as well as additional basic functions [2]. Thymol, carvacrol and c-terpinene antioxidant properties have been accounted for previously. Consequently, essential oil movement can be attributed to the high content of these components. Furthermore, essential oil components are more effective than free radical scavenging in preventing conjunctivitis [3]. Trans-resveratrol and quercetin are non-flavonoid and flavonoid polyphenols, each of which has higher concentrated antioxidant action than vitamins E and C [4]. Some antioxidants cannot be produced by the body as micronutrients, for example, vitamin E, β-carotene and vitamin C, and therefore need to be improved in the regular diet [5].

Inflammation is an irregular process, the expansion of vascular penetrability, increase of protein denaturation and membrane stabilization. The body cells are harmed by physical or chemical mediators; the injury is in the system stress [6]. At the beginning of inflammation, the cells release inflammatory mediators. These mediators contain histamine, serotonin, slow responding substances of anaphylaxis (SRS-A), prostaglandins and some coagulating system, the fibrinolytic system and the kinin system [7]. A large number of flavonoids have been demonstrated to be potential immunomodulators, acting as anti-inflammatory, anti-stress, anticancer operators and various skin diseases [8]. The healing capability of flavonoids and the need for scientific approval in well-known medication have incited expanded enthusiasm for the field.

Banana is a tropical fruit grown in more than 122 countries around the world [9]. As of 2004, the developed zone covers 3.8 million hectares and is the fourth-largest producer of 56.4 million metric tons of heavy fruit, after rice and corn and milk [10]. Banana peels have general anti-inflammatory, antiseptic, cooling properties that help reduce the severity of side effects. Crops span 11-14 months. Just as bananas are packed with supplements, their peels additionally pack a healthy punch. The banana peel contains more fiber and potassium than banana material. Banana peel contains vitamins such as vitamin A, vitamin B6 minerals such as calcium, manganese, magnesium, sodium and sulphur. Banana peel contains antioxidants besides. Banana peel can protect our heart, reduce skin aging, relieve eczema and psoriasis, reduce weight and cancer cure.

MATERIALS AND METHODS

Collection of banana Peel The peel of banana was collected from the local market of Kakinada, Andhrapradesh the samples were properly authenticated by senior botanist and taxonomist.

Preparation of Peel Material

Rinse the peels with tap water and let dry in the shade (15 days) until completely dry. Then cut it into small pieces, ground them coarsely and should be store at room temperature.

Extraction with Solvent

Dry samples were subjected to extraction using solvent ethanol. A 10 g powder sample with 100 ml ethanol was collected in a conical flask while shaking. The extract was transferred to pre-weighed glass vials. This process was repeated three times in fresh solution using the same substance.

Chemicals

1,1-diphenyl-2-picrylhydrazyl(DPPH), ethanol, folin-ciocalteu's reagent, sodium carbonate, Gallic acid, Quercetin, Aluminium chloride, potassium acetate, DMSO, H₂O₂, Ascorbic acid, diflofenac sodium, sodium chloride, sodium citrate, dextrose distilled water.

Phytochemical screening

Qualitative phytochemical screening

The ethanol extract of banana peels was analysed for flavonoids, alkaloids, steroids, phenols, carbohydrates, proteins, tannins, saponins and glycosides using standard procedures.

Quantitative Phytochemical Estimation

Determination of Total Phenol Content

Folin-ciocalteu's reagent method:

The complete phenol content in the ethanol extract of Banana peels was dictated by the reagent strategy for foliocaltium [11]. 0.5ml of concentrate and 0.1ml (0.5N) folin-ciocalteu's reagent blend was incubated at room temperature for 15mins then 2.5ml immersed sodium carbonate solution was included and further incubated for 30mins at room temperature and the absorbance was estimated at 760nm. Gallic acid was utilized as a positive control. The absolute phenolic value is communicated regarding gallic acid equivalent (mg/g of extracted compounds).

Determination of Total Flavonoid Content

Aluminium chloride calorimetric method:

The flavonoid substance of the ethanol extract of banana peels was dictated by the aluminum chloride calorimetric technique [12]. The reaction blend was incubated at 1 mL (1 mg/ml) and 0.5 mL (1.2%) of aluminum chloride and 0.5 mL (120 mM) of potassium acetate derivation at room temperature for 30 min.

The absorption of all samples is estimated at 415nm. The flavonoid content is communicated regarding quercetin equivalent (mg/g of the extracted compound). Quercetin has been utilized as a positive control.

EVALUATION OF ANTIOXIDANT ACTIVITY

Radical scavenging activity (RSA) DPPH assay:

DPPH radical scavenging activity was assessed by spectrophotometric procedure [13]. 10mg of test extract was disintegrated in 1ml of DMSO. The sample extract was added at various concentrations included (100,200,300,400 and 500 µg) with 2.96 ml of DPPH solution in dark condition. It incubates for 20 minutes. Absorption was noted at 517nm. The percent radical scavenging activity was calculated utilizing the following Eqn. 1,

$$\% \text{ inhibition} = A_{\text{control}} - A_{\text{sample}}/A_{\text{control}} \times 100$$

Where A_{control} was the absorbance of the control and A_{sample} was the absorbance of the sample.

Hydrogen peroxide (H₂O₂) scavenging activity

The scavenging activity of H₂O₂ was assessed by banana peel extract with minor adjustment utilizing the strategy for Ruch et al [14]. A 4 mmol / L solution of H₂O₂ was formed at PBS (pH 7.4). Peel extract (4 mL), prepared in distilled water at different concentrations was blended in with 0.6 mL of 4 mmol/L H₂O₂ solution arranged in PBS and incubated for 10 min. The solution was absorbed at 230 nm against the blank solution containing the plant extract in PBS without hydrogen peroxide. Ascorbic acid has been utilized as a positive control. The amount of H₂O₂ radical inhibited by the extract was calculated using the following Eqn. 2,

$$\text{H}_2\text{O}_2 \text{ radical scavenging activity} = (A_{\text{control}} - A_{\text{test}})/A_{\text{control}} \times 100$$

Where, A Control is the absorbance of H₂O₂ radical+ethanol; Atest is the absorbance of H₂O₂ radical+sample extract or standard.

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

Inhibition of protein denaturation

The reaction mixture consisted of 0.45 ml of bovine serum albumin (5% aqueous solution) and 0.05 ml of banana peel extract in various concentrations. Heated at 56 °C for 3 min and incubating at 37 °C for 30 min. After 2.5 ml of phosphate buffer solution (pH 6.3) was added into each test tube. Turbidity was measured spectrophotometrically at 640 nm. Distilled water (0.05 ml) was used instead of extracts as control tests; while product control tests required bovine serum albumin [15]. The percent anti-inflammatory activity was calculated utilizing the following Eqn.3

$$\% \text{ inhibition} = (\text{abs}_{\text{control}} - \text{abs}_{\text{test}})/\text{abs}_{\text{control}} \times 100,$$

Where, $\text{abs}_{\text{control}}$ means absorbance of control sample; abs_{test} means absorbance of test sample

RESULTS AND DISCUSSION

Phytochemical analysis of ethanol extract of banana peel

Qualitative screening:

Various chemical tests are performed and are given in **Table 1** Basic phytochemical tests of banana peel showed the presence of flavonoids, alkaloids, steroids, phenols, carbohydrates, proteins, tannins, saponins and glycosides.

Table 1: qualitative phytochemical screening

Bioactive compounds	Amounts µg/ml
Total phenol	149.9
Total flavonoids	138.7

+ indicates Presence of phytochemical constituents

Quantitative screening:

The result of total phenol and flavonoid content from the given **Table 2** phenol content was found to be higher than flavonoid content in ethanol extract of Banana peel.

Table 2: quantitative phytochemical screening

Phytochemicals	Ethanol extract
Flavonoids	+
Alkaloids	+
Steroids	+
Glycosides	+
Proteins	+
Phenols	+
Saponins	+
Tannins	+
Carbohydrates	+

ANTI-OXIDANT ACTIVITY:

DPPH radical scavenging activity

Banana peel extract showed significant activity in DPPH radical scavenging activity and showed a maximum effect on ethanol extract compared to standard ascorbic acid as shown in **Table3:**

Table 3: DPPH radial scavenging activity assay of ethanol extract of banana peel

Sr.No	Concentration µg/ml	% inhibition on DPPH	
		Ethanol extract	Standard ascorbic acid
1	100	33.48	45.27
2	200	42.72	57.09
3	300	60.63	74.34
4	400	81.07	89.05
5	500	94.15	97.01

DPPH-1, 1-diphenyl-2-picrylhydrazyl

Hydrogen peroxide assay

Banana peel extract showed significant activity in the H₂O₂ method and showed a maximum effect on ethanol extract when compared to standard ascorbic acid as shown in **Table 4**:

Table 4: hydrogen peroxide scavenging activity assay of ethyl acetate extract of banana peel

Sr.No	Concentration µg/ml	% inhibition on H ₂ O ₂ assay	
		Ethanol extract	Standard ascorbic acid
1	100	32.00	43.09
2	200	40.72	51.83
3	300	57.89	69.31
4	400	79.27	80.65
5	500	92.13	95.04

H₂O₂-hydrogen peroxide

ANTI-INFLAMMATORY ACTIVITY:

Banana peel extract showed significant activity on the protein denaturation method in ethanol extract when compared to standard diclofenac sodium. The following result of percentage inhibition of ethanol extract of banana peel is shown in **Table 5**.

Table 5: protein denaturation method of ethyl acetate extract of banana peels

Sr.No	Concentration µg/ml	% stabilization on protein denaturation method	
		Ethanol extract	Standard Diclofenac Sodium
1	100	23.73	37.00
2	200	35.32	49.56
3	300	50.45	62.45
4	400	69.20	73.34
5	500	78.22	87.89

Plant extracts are an important resource for the development of new therapeutic agents. Phenolic acids and flavonoids have biological activities, including anti-inflammatory, anti-cancer. These activities may be related to their antioxidant activity [16]. The antioxidant action of flavonoids reduces the formation of free radicals and eliminates free radicals. Phenolic compounds are important plant antioxidants that have demonstrated significant scavenging activity against radicals. Therefore the antioxidant capacity of a sample is mainly due to its phenolic compounds [17]. Hence the antioxidant, anti-inflammatory, potential of banana peel extract may be due to the presence of high phenolic compounds in it. DPPH radical scavenging activity test from the dosage-dependent response curve of DPPH's radical scavenging activity of banana ethanol extract, ethanol extract has a high radical scavenging activity with a concentration of 500 µg/ml. The maximum percentage reached 94.15% (500 µg / ml), which is comparable to the standard drug ascorbic acid 97.01% (500 µg / ml) as shown in **Fig. 1**. The H₂O₂ scavenging action of ethanol extract of banana peel has been studied to be effective at 92.13% (500 µg/ ml) as shown in **Fig. 2**. The protein

denaturation test results of the ethanol extract of banana peel were shown to be effective at 78.22% (500 g / ml) compared to the standard drug diclofenac sodium 87.89% (500µg / ml) as shown in Fig.3.

Figure 1: percent inhibition on DPPH radical scavenging activity assay using ethanol Extract of banana peel

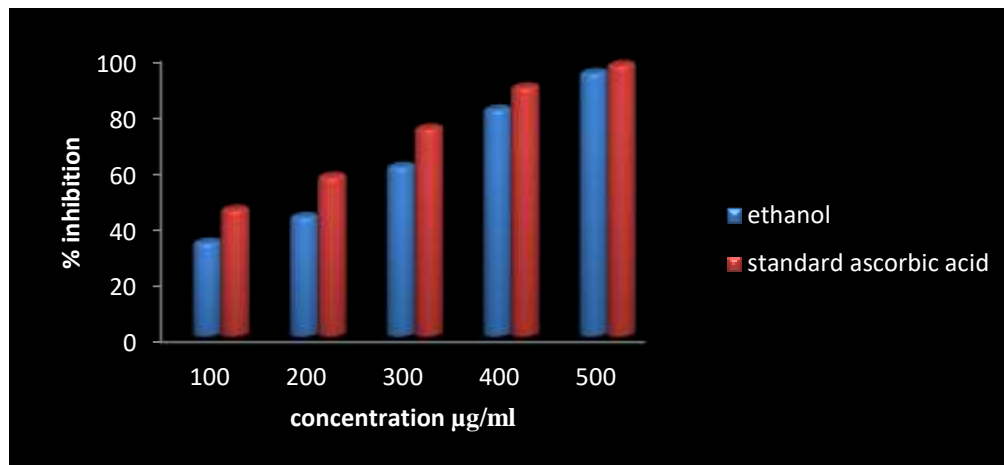


Figure 2: percent inhibition on H₂O₂ radical scavenging activity assay using ethanol extract of banana peel

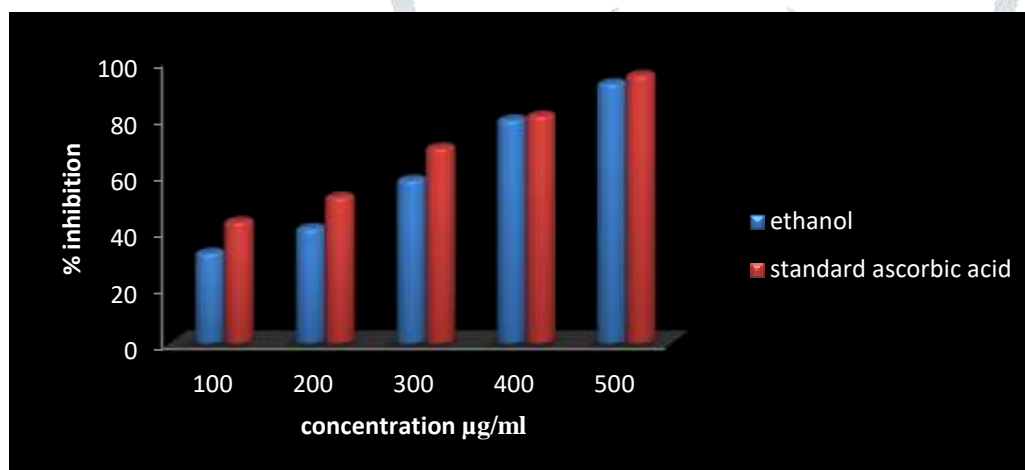
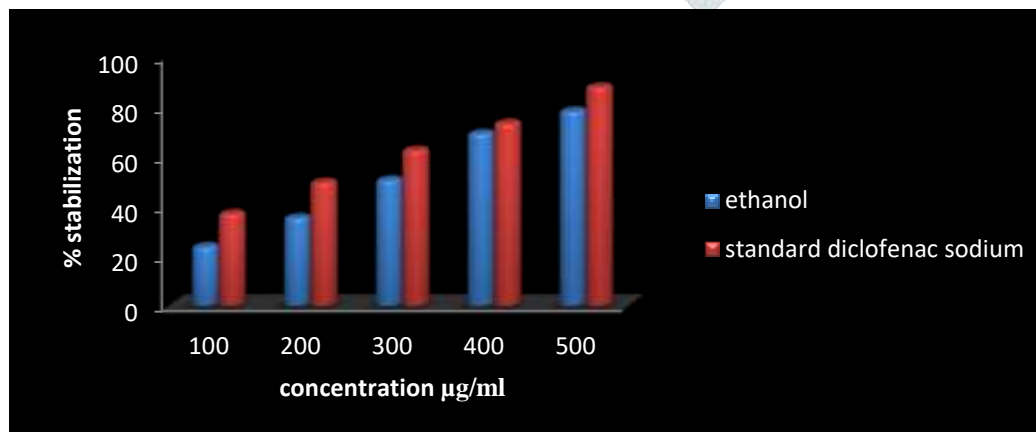


Figure3: percent stabilization on protein denaturation assay using ethanol extract of banana peel



CONCLUSION

The result of the study revealed that these phytochemicals represent the potential for banana peel, as it contains large amounts of phenols and flavonoids. Further mechanical studies are needed to isolate, purify and analyse the specific bioactive compound for antioxidant activity.

The anti-inflammatory action of banana peel significantly prevents membrane lysis resistance. This suggests that banana peel can be considered an effective source of anti-inflammatory bioactive compounds.

REFERENCE

1. Kunwar A, Priyadarsini KI. Free radicals, oxidative stress and importance of antioxidants in human health. *Journal of Medical & Allied Sciences*. 2011 Jul 31;1(2):53.
2. Lander HM. An essential role for free radicals and derived species in signal transduction. *The FASEB journal*. 1997 Feb;11(2):118-24.
3. Ruberto G, Baratta MT. Antioxidant activity of selected essential oil components in two lipid model systems. *Food chemistry*. 2000 May 1;69(2):167-74.
4. Stojanović S, Sprinz H, Brede O. Efficiency and mechanism of the antioxidant action of trans-resveratrol and its analogues in the radical liposome oxidation. *Archives of Biochemistry and Biophysics*. 2001 Jul 1;391(1):79-89.
5. Teresa MM, Magdalena W, Andrzej K. A systematic review of the effect of vitamin C infusion and vitamin E-coated membrane on hemodialysis-induced oxidative stress. In *Hemodialysis-Different Aspects* 2011 Nov 14. IntechOpen.
6. Leelaprakash G, Dass SM. In vitro anti-inflammatory activity of methanol extract of *Enicostemma axillare*. *International Journal of Drug Development and Research*. 2011 Jul;3(3):189-96.
7. Perianayagam JB, Sharma SK, Pillai KK. Anti-inflammatory activity of *Trichodesma indicum* root extract in experimental animals. *Journal of ethnopharmacology*. 2006 Apr 6;104(3):410-4.
8. Crozier A, Jaganath IB, Clifford MN. Dietary phenolics: chemistry, bioavailability and effects on health. *Natural product reports*. 2009;26(8):1001-43.
9. Husain MD, William R. Status of banana cultivation and disease incidences in Malaysia. *Crop Protection and Plant Quarantine Division, Department of Agriculture, Malaysia*. 2010;60.
10. Chai M, Ho YW, Liew KW, Asif JM. Biotechnology and in vitro mutagenesis for banana improvement. *Banana Improvement: Cellular, Molecular Biology and Induce Mutations*, Jain, S. and R. Swennen (Eds.). Science Publisher Inc., USA. 2004:59-77.
11. Atmani D, Chaher N, Berboucha M, Ayouni K, Lounis H, Boudaoud H, Debbache N, Atmani D. Antioxidant capacity and phenol content of selected Algerian medicinal plants. *Food Chemistry*. 2009 Jan 15;112(2):303-9.
12. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis*. 2002 Jul 1;10(3).
13. Sreejayan N, Rao MN. Free radical scavenging activity of curcuminoids. *Arzneimittel-forschung*. 1996 Feb;46(2):169.
14. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*. 1989 Jun 1;10(6):1003-8.

15. Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Membrane stabilizing activity—a possible mechanism of action for the anti-inflammatory activity of Cedrus deodara wood oil. *Fitoterapia*. 1999 Jun 1;70(3):251-7.
16. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. *Critical reviews in food science and nutrition*. 1998 Aug 1;38(6):421-64.
17. Wei SD, Zhou HC, Lin YM. Antioxidant activities of extract and fractions from the hypocotyls of the mangrove plant *Kandelia candel*. *International journal of molecular sciences*. 2010 Oct;11(10):4080-93.

