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EVALUATION OF THE *INVITRO* ANTIOXIDANT EFFECT OF *ANOGEISSUS ACUMINATE*

Sankar V.[1]*, Anand Babu K.[2], Ragu S.[1] and Tamilselvan A.[1]

[1]Department of Pharmacology, Vinayakamission's College of Pharmacy, Vinayaka Missions University, Salem-636008.

[2]Department of Pharmaceutical Chemistry, Sri Ramachandra University, Chennai -600116.

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***Corresponding Author**
Sankar V.
Department of Pharmacology, Vinayakamission's College of Pharmacy, Vinayaka missions University, Salem-636008.

ABSTRACT

The present study was aimed to investigating the antioxidant activity of the ethanolic extract of *Anogeissus acuminate* was studied by using different *invitro* methods such as DPPH scavenging assay and Hydrogen peroxide scavenging (H_2O_2) assay. The ethanolic extract of the plant 500µg/ml had shown potent activity in DPPH assay and Hydrogen peroxide scavenging assay. The findings suggest that the ethanol extract of *Anogeissus acuminate* is a effective free radical scavenger, augmenting its therapeutic value.

KEYWORDS: *Anogeissus acuminate*, DPPH, H_2O_2, Free radical, Antioxidant.

INTRODUCTION

In human life oxygen is very essential, without oxygen we cannot survive. Our evolutionary ancestors developed defense mechanisms that can minimize the toxic effects of oxygen. A free radical is defined as any atom or molecule possessing unpaired electrons.[1] Antioxidants are agents which scavenge the free radicals and prevent the damage caused by reactive oxygen species (ROS)and reactive nitrogen species (RNS). ROS is composed of superoxide anion (O_2), hydroxyl H), hydroperoxyl (OOH), peroxyl (ROO·), alkoxyl (RO·) radicals.[2] Free radicals are responsible for causing a large number of diseases including cancer,[3] cardiovascular disease and neural disorders.[4] Free radicals induced by peroxidation have gained much importance because of their involvement in several hydroxyl pathological conditions. Reactive oxygen species (ROS) such as superoxide anions (O2.), radical (OH.) and nitric oxide (NO) inactivate enzymes and damage important cellular components causing

injury through covalent binding and lipid peroxidation .*Anogeissus acuminate is* known as Button tree. It is a deciduous tree with a narrow crown; it can grow up to 40 meters tall the long, straight bole is un buttressed and can be 100cm in diameter widely used in skin diseases like eczema, dermatitis, skin ulcers and anti-inflammatory and analgesic activity. The present study will be discussed about invitro antioxidant effect of the plant.[4]

MATERIALS ANDMETHODS

Plant material: The plant *Anogeissus acuminate* was collected from Tirupathi hills, Andhra Pradesh. The plant was taxonomically identified and authenticated by the Botanist Dr.V.Chelladurai, The authenticated plant material was used for the preparation of extracts.

Preparation of plantextract[5]

The leaves of this plant were dried under shade at 27-30°C for 15-30 days, after which the leaves of the plant were chopped and grounded into coarse powder. The powder (400 g) was extracted with ethanol (1500 ml) overnight, at room temperature with constant stirring. The extraction was carried out by continuous hot percolation using soxhlet apparatus. The extract was filtered and the filtrate was concentrated at 30°C under reduced pressure in a rotary evaporator. The yield (w/w) of the crude extract was found to be 83.08%. .Phytoconstituents present in the various extracts were identified by chemical tests shows high phenolic and flavonoids contents.

Drugs and chemicals

All the drugs and chemicals used in the study were obtained commercially and were of analytical grade.

Chemicals used

Ethanol, KH_2PO_4 buffer, Hydrogen Peroxide, Ascorbic Acid., DPPH

1. Hydroxyl radical scavengingassay[6]

Hydroxyl radical scavenging activity was measured by the ability of the extract to scavenge the hydroxyl radicals generated by the Fe^{3+}- Ascorbate-EDTA-H_2O_2 system (Fenton reaction). The reaction mixture in a final volume of 1.0 ml contained 100 µl of 2-deoxy2-ribose (28 mm in 20 mm KH_2PO_4 buffer, pH 7.4), 500µl of the fractions at various concentrations (50,100,500 µg/ml) in buffer, 200 µl of 1.04 mm EDTA and 200 µM $FeCl_3$ (1:1v/v), 100 µl of1.0 mm hydrogen peroxide (H_2O_2) and 100 µl of 1.0 mm ascorbic acid

ferrous chloride (0.1 ml) and 5 mm ferrozine (0.2 ml) to initiate the reaction and the mixture is shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the solution is measured at 562nm.Theascorbic acid is used as a positive control and all tests and analysis were run in triplicate. The percentage chelating effect of Ferrozine-Fe^{2+} complex formation was calculated by using the following formula.

Calculation of percentage inhibition(%I)[7]

The concentration (g/ml) of the extract required to scavenge the radicals was calculated by using the percentage scavenging activities at three different concentrations of the extract. Percentage inhibition (I %) was calculated using the formula, I % = (Ac-As) x100

2.DPPH radical scavengingactivity[8]

DPPH scavenging activity or the Hydrogen donating capacity was quantified in presence of stable DPPH radical on the basis of Blois method. Briefly, to a methanolic solution of DPPH (100µM, 2.95ml), 0.05ml of test compounds dissolved in methanol was added at different concentration(50,100, 500µg/ml). Reaction mixture was shaken and absorbance was measured spectrophotometrically at 517 nm at regular intervals of 30seconds for 5 minutes, and the reading was taken till 20 min. Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated by using the following equation. The results were depicted in Table no.4

Scavenging effect (%) =(1-B/A)X100

Where,

A=Absorbance of DPPH control with solvent (517nm)

B=Absorbance of decolorized DPPH in presence of test sample (517nm)

RESULTS AND DISCUSSION

1.Hydroxyl radical scavenging activity

Ascorbic acid and *Anogeissus acuminate* showed hydroxyl radical scavenging activity with about 34.18-84.31% at concentration of 50,100and 500 µg/ml *(*Table 1*).* A concentration dependent inhibition against hydroxyl radical induced deoxy ribose degradation was observed in the deoxy ribose assay. Because the *Anogeissus acuminate*has high phenol and flavonoid content, its antioxidant compounds may well act as antioxidant and scavenge hydroxyl radical generated from the Fenton reagent

Table No: 1 Hydrogen peroxide-scavenging activity of *Anogeissus acuminate*

S.No	Concentration (µg/mL	%Radical scavenged	
		Ascorbic acid	Ethanolic extract of *Anogeissus acuminate*
1	50	34.18	40.12
2	100	64.13	55.64
3	500	84.31	80.75

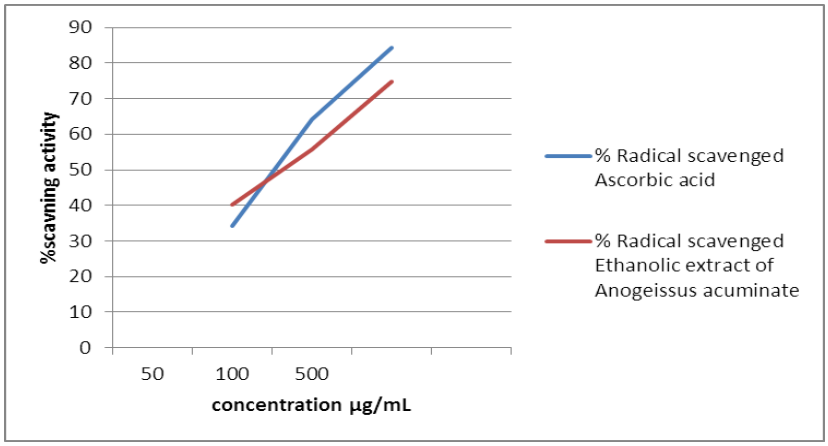

Fig no: 1 Effect of ethanolic extract of *Anogeissus acuminate* by hydrogen peroxide scavenging Activity

2. DPPH Radical Scavenging Activity[9]

The DPPH radical scavenging potential of the compound or extract is determined by measuring the decrease in absorbance of DPPH radicalat517nm as a result of the formation of its reduced form. The DPPH free radical scavenging activity of the extract was carried out. The extract were tested at concentrations of50, 100, 500µg/mL. The plant extract has shown82.34%inhibitionof theDPPHradicalat500µg/mL concentration, where as the standard (Ascorbic acid) hasshown86.74%inhibitionatthesameconcentration. This study has the similarity with previous investigation Finally the extract of *Anogeissus acuminate* exhibited significant antioxidant activities against DPPH radical scavenging activity and reducing power assay; however, activity shown by ethanolic extracts was maximum. The reported antioxidant activity may be due to the presence of phytochemicals in the titled plant.

Table: 2DPPH - scavenging activity of *Anogeissus acuminate*

S.No	Concentration (µg/mL)	%Radicalscavenged	
		Ascorbic acid	Ethanolic extract of *Anogeissus acuminate*
1	50	41.02	40.1
2	100	73.2	68.72
3	500	86.74	82.34

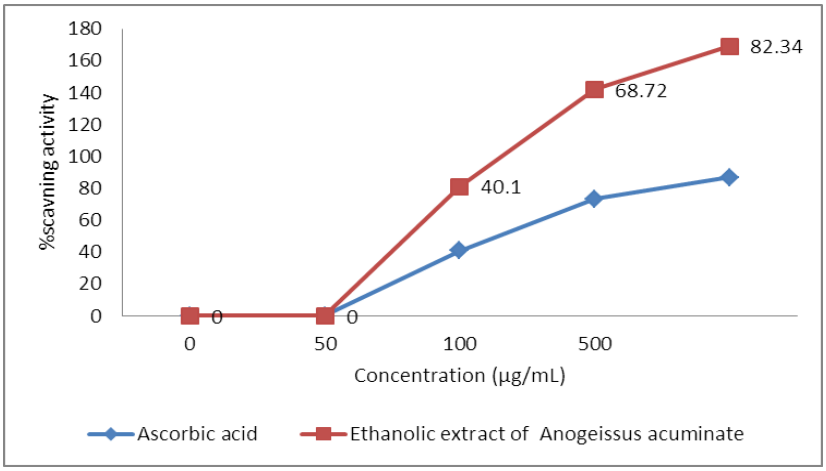

Fig No: 2Effect of Ethanolic Extract of *Anogeissus Acuminate* By DPPH Method

CONCLUSION

Based on the results obtained, *Anogeissus acuminate* was showed potent antioxidant and free radical scavenging activity not remarkably different than reference compound ascorbic acid. Major antioxidative component seems to be phenolic and flavonoids. Therefore, it can be concluded that the ethanolic extract of *Anogeissus acuminate* could be considered for prevention and treatment of human diseases and its complications as potent antioxidant.

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