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IN VITRO DETERMINATION OF ANTIOXIDANT ACTIVITY OF *PHYSALIS ANGULATA* LNN.

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ABSTRACT

The present investigation was carried out to determine the antioxidant properties in methanolic extract of leaves, stem, fruit and roots of *Physalis angulata* (L). The antioxidant assay was carried out using free radicals DPPH, superoxide, nitric oxide, hydrogen peroxide and hydroxyl radical. Among the extracts tested fruit and leaf extracts were found to be significant in their antioxidant property than stem and roots. However, amounts of total phenolic and flavanoid content were high in leaves and fruit extracts.

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KEYWORDS:Total phenolic compounds, DPPH, superoxide, nitric oxide, hydrogen peroxide and hydroxyl radical.

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INTRODUCTION

Reactive Oxygen Species (ROS) are highly reactive molecules which form free radicals by the help of molecular oxygen. These free radicals are also produced in aerobic species during the process of mitochondrial electron transport chain. These are potential to cause deleterious effects in the living system. [1] Among, the free radicals RNS (Reactive nitrogen species e.g. nitric oxide NO) is an essential intermediate of metal catalyzed oxidation reactions.[2] However, the excess generation of RNS leads to nitrosative stress.[3,4] This condition makes it difficult to neutralize and eliminate the free radicals. The reactive oxygen species and other free radicals are readily neutralized either by enzymatic or non-enzymatic processes. [5] In current scenario majority of the population are habituated to depend on the traditional medicine to reduce hazardous effects generated by the ROS. Generally, medicinal plants are considered to be major source of natural drugs. [6]. These naturally derived drugs are much more potent in preventing various human disorders produced due to oxidative stress, which includes atherosclerosis, ischemic cardiac attacks, diabetes, heaptotoxicity, inflammation, immunosupression, ageing, neurological disorders [7,8].There are a considerable number of natural products used in the traditional medical systems in many countries as alternative medicine for the treatment of various diseases[9]. Many of these medicinal plants provide relief of symptoms comparable to that obtained from allopathic medicines[9]. One such plant is *Physalis angulata*, a branched annual shrub is commonly known (in Brazil) as camapu or balaozinho belonging to Solanaceae [10]. This plant is widely distributed throughout tropical and subtropical regions of the world. Extracts or infusions of this plant have been used in many countries for the treatment of a variety of diseases viz., hepatitis, asthma, malaria, dermatitis and rheumatism [11-13]. It is also reported that physalins (A, B, D and F) and glycosides such as Myricetin-3-O-neohesperidoside which were isolated from organic fractions of *Physalis angulata* show significant anticancer activities on various cancer cell lines such as HeLa (cervix uteri), HA22T (hepatoma), leukemia, lung adenocarcinoma and epidermoid carcinoma of the nasopharynx KB-16 cell lines [14-15,13].The present investigation is carried out for the evaluation of antioxidant activities of various parts of this plant viz., leaves, stem, fruits and roots.

MATERIALS AND METHODS

Chemicals

Sulphanilamide, Folin- Ciocalteu reagent, Sodium nitroprusside, Nitro blue tetrazolium (NBT), tannic acid were procured from SRL Mumbai. 1,1-diphenyl-1- picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich Chemical Co,. Other chemicals and reagents were purchased are of research grade.

Plant material

Leaves, stem, fruits and roots of physalis angulata were collected during August to November, from the fields nearer to Dharmasagar, district Warangal, Andhra Pradesh, India. Species authentification was done by Prof. V. S. Raju Taxonomist, Department of Botany, Kakatiya University, Warangal.

Extraction procedure

All the parts excpet fruits were chopped, shade dried and grinded in homogenizer in to coarse powder. Fruits were initially processed for the removal of seeds, shade dried and grinded in homogenizer in to coarse powder. The 100 g of each powdered material was extracted with methanol and concentrated under rotavapour at 40-50^{o}C.

Reagents preparation

Preparation of Nash Reagent: 75.0 g of ammonium acetate, 3 ml of glacial acetic acid

and 2 ml of acetyl acetone were mixed and distilled water was added to total volume of 1 L.Preparation of Griess Reagent: 1% sulphanilamide, 2% Phosphoric acid and 0.1% N-1- napthylethylenediamine dihydrochloride in distilled H_2O.Preparation of Ferrous EDTA: 0.13% ferrous ammonium sulfate and 0.26% EDTA in distilled H_2O.

Determination of total phenol content

The amount of total phenolics in the extracts was determined with Folin–Ciocalteu reagent as described.[16] To, 1 ml of each methanolic extract of various parts of *Physalis angulata* at different concentrations (100, 200 and 300 µg/ml) and standard solution of tannic acid (5 and10 µg/ml) were added to 100 ml volumetric flask separately, that contained about 60 ml distilled water and followed by the addition of 5 ml of Folin– Ciocalteu reagent. The content was mixed thoroughly and kept constant for about 10 min. To this, 15 ml Na_2CO_3 (20 %) was added and made up to 100 ml using distilled water. The mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm using a UV-visible spectrophotometer. The content was expressed as equivalent of tannin (µg) by using the following equation, which was obtained from a standard tannic acid graph. Absorbance = 0.001 × tannic acid (µg)+0.0033.

Estimation of total flavonoid content

Flavonoid content was measured by aluminum chloride colorimetric assay with slight modification[17]. 1 ml if each methanolic extracts of various parts of *Physcalis angulata* with different concentrations (100, 200 and 300 µg/ml) and standard solution of catechin (5 and10 µg/ml) was added separately to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5% $NaNO_2$ was added, followed by the addition of 0.3 ml of 10% $AlCl_3$ after 5 min. After incubation period of 6 min 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm.

Anti-oxidant assay

DPPH radical scavenging activity

Free radical scavenging capacity of various parts of *Physalis angulata* was determined by using DPPH[18]. DPPH radical scavenging activity was done by serial dilution by taking diluted methanol (1:20) as standard. 10 ml of various diluted methanolic extracts of various concentrations (100, 200 and 300 µg/ml) were added to 1 ml DPPH solution (0.004%) and incubated for 10 min at room temperature. Absorbance of test and reference standard, ascorbic acid was measured at 517 nm. The amount of DPPH scavenging was calculated by using the formula:

% DPPH radical scavenging = [(Absorbance of control – Absorbance of test sample)/ (Absorbance of control)] × 100

Super oxide radical scavenging activity

The super oxide radical scavenging activity was measured by the following described method.[19-20] 1ml of each methanolic extracts of various parts of *physalis angulata* at various concentrations (100, 200 and 300 µg/ml) were mixed with 1 ml of nitro blue tetrazolium (NBT) solution (156 mM NBT in phosphate buffer of pH 7.4) and 1 ml NADH in phosphate buffer of pH 7.4. The reaction was initiated by adding 100 ml of phenazine methosulfate (PMS) solution (60 mM PMS in phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25^0C for 5 min and the absorbance was measured at 560 nm against blank sample and compared with reference standard ascorbic acid. Decreased absorbance of reaction mixture indicated increased superoxide anion scavenging activity. The percentage of inhibition of superoxide anion

generation was calculated using the following formula:

% inhibition = [(Absorbance of control – Absorbance of test sample)/ (Absorbance of control)] × 100

Nitric oxide scavenging activity

Nitric oxide scavenging activity was determined by the following method described by.[21] Briefly, 5 mM sodium nitroprusside was prepared in

phosphate buffered saline and mixed with different concentrations of methanolic of various parts of *Physalis angulata* at (100, 200 and 300 µg/ml) followed by incubation at 25^0C for 30 min. A control without the extracts but with equivalent amounts of methanol was taken. After 30 min of incubation1.5 ml of solution was pipptted out and diluted with 1.5 ml of Griess reagent. Absorbance of chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1- napthylethylenediamine dihydrochloride was measured at 546 nm and percentage scavenging activity was measured with reference standard ascorbic acid.

Scavenging of hydrogen peroxide

Scavenging of hydrogen peroxide was measured by the following described method elsewhere.[22] A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). 1 ml of each methanolic extracts of various parts of *Physalis angulata* at different concentrations (100,200 and 300 µg/ml) were added to 0.6 ml of 40 mM hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of plant extract and reference standard ascorbic acid was calculated using the following formula:

% scavenged [H_2O_2] = [(Absorbance of control – Absorbance of test sample)/ (Absorbance of control)] × 100

Hydroxyl radical activity

Hydroxyl radical activity was measured by the following described method elsewhere.[23] 1 ml of each methanolic extract of various parts of physalis angulata at various concentrations (100,200 and 300 µg/ml) were placed in tubes and evaporated to dryness. 1 ml of ferrous-EDTA (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5ml of 0.018% EDTA, 1 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) and 0.5 ml of freshly prepared 0.22% ascorbic acid were added to each tube. The tubes were capped tightly and heated in a water bath at 80-90^0C for 15 min. Reaction was terminated by addittion of 1 ml ice cold TCA (17.5% w/v) and followed by addition of 3 ml of Nash reagent and left at room temperature for 15 min. The intensity of color formed was measured at 412 nm against the reagent blank. The percentage inhibition was compared with reference standard ascorbic acid and test compounds.

Statistical analysis

The data from the experiments of antioxidant activity are presented as mean ± S.E.M (n=3). Student's *t*-test was used for statistical analysis (SAS software 9.0). Values were considered statistically significant when $P<0.5$.

RESULTS AND DISCUSSION

The methanolic extracts of *Physalis angulata* leaf, stem, fruit and root had fairly noticed good percentage of total phenolic compounds at all tested concentrations. The highest percentage observed with fruit methanolic extract 300 µg/ml was 84 % followed by, leaf extract with 78%. Standard tannic acid tested at 5 and 10 µg/ml produced 79% and 92% respectively (Fig. 1). The total flavanoid content was significantly noticed high at 300 µg/ml in fruit and stem methanolic extracts compared to reference standard catechin. The yield percentage of these extracts was recorded as 79% and 71% respectively (Fig. 2). Synthetic antioxidants are avoided because of their adverse effects while, the natural plant extracts are preferred due to their property of good antioxidants.[24] In this pertinent, we have been investigated few fractions of *physalis angulata* various parts for the screening of antioxidant properties. It was observed that all the extracts showed concentration dependent scavenging activity. Among the extracts tested the highest antioxidant activity was exhibited by fruit and leaf extracts. 1,1-diphenyl-2-picrylhydrazyl (DPPH) which is a common free radical used for the evaluation of reducing substances. The scavenging activity of DPPH radical was determined by the reduction in the absorbance because of change in color from purple to

yellow. In the present investigation, the methanolic extracts of fruit and leaves extract were notably, exhibited scavenging activity compared to other extracts. The values were obtained with fruit and 0.43±0.25 and 0.25±0.14 which were significant to $p<0.5$ and are noticed at 300 µg/ml concentration of extract (Table. 1). Generally, the superoxide anion radicals generated by enzymes like xanthine oxidase is further converted to hypoxanthine and in turn to uric acid.[25] The decrease in absorbance at 560 nm is a direct indication of the utilization of superoxide anion in the reaction mixture and build up relation of concentration dependent superoxide scavenging activity. In the current studies revealed that the methanolic fruit and leaf extracts reported significant scavenging activity than other extracts. The values of fruit and leaf extracts which are obtained at 300 µg/ml are 0.36±0.20 and 0.3±0.17 respectively, which were comparable to reference standard. The value of fruit extract is set at $p<0.5$ (Table.1). Nitric oxide (NO), acts as a potent pleiotropic mediator which involve in several physiological processes undergo interaction with superoxide anion for the formation of peroxynitrous acid (ONOOH) which is potentially cytotoxic molecule, and proves to be strong oxidant and that undergoes the nitration of aromatic compounds such as tyrosine[17]. Peroxynitrite ($ONOO^-$) on the combination of CO_2 is dissolved in body fluids is more potent to oxidize proteins in living systems.[26-27] Among the extracts tested fruit and leaf extracts produced satisfactory results. The p-value for the inhibition of nitric oxide is considered to be <0.5. In the present study the values of fruit and leaf extracts which were obtained at 300 µg/ml concentration are 0.45±0.26 and 0.34±0.2 respectively (Table.1). H_2O_2 is considered as weaker oxidizing and reducing agent. However, it acts as good toxicant, when it undergo an interaction with metal ions and superoxide anion which converts itself into hydroxyl radical which leads to generate a single oxygen. These H_2O_2 molecules undergo lysis of heamoglobin, to release Fe ions.[28] Thus, it is important to determine the ability of scavenging activity of H_2O_2 of the plant extracts. Interestingly, all the plant notably exhibited scavenging activity. However, fruit is proved to possess highest scavenging activity than others. The value of fruit extract obtained at 300 µg/ml is 0.11±0.06 (Table.1). Hydroxyl radical generated during the Fenton's reaction participates in the destruction mechanism of base pairs from DNA molecule.[29] The methanolic extracts of fruit, holds more scavenging activity compared to other extracts. This might be because of the nature of generated free radical and its physical and chemical differences occurring as antioxidant by the both plant extracts.[30-31] However, the mechanism of the reaction of stable and non-stable free radicals may differ with a particular antioxidant. In the case of stable free radical DPPH which react stoichometrically with the antioxidants are good hydrogen donors. Whereas, antioxidants which are very effective as chelators of transition metal ions undergo the reaction in different manner in the scavenging activity of hydroxyl radical when compared to assays with stable free radical. The values which were noticed at 300 µg/ml concentration of fruit and leaf extracts are 0.34±0.2, 0.36±0.20, respectively (Table. 1)This appears to be the first report of antioxidant activity of *Physalis angulata* plant.

Figure 1

Yield percentage of total flavanoid content (100 g) of various parts of Physalis angulata at various concentrations compared to reference standard catchien.

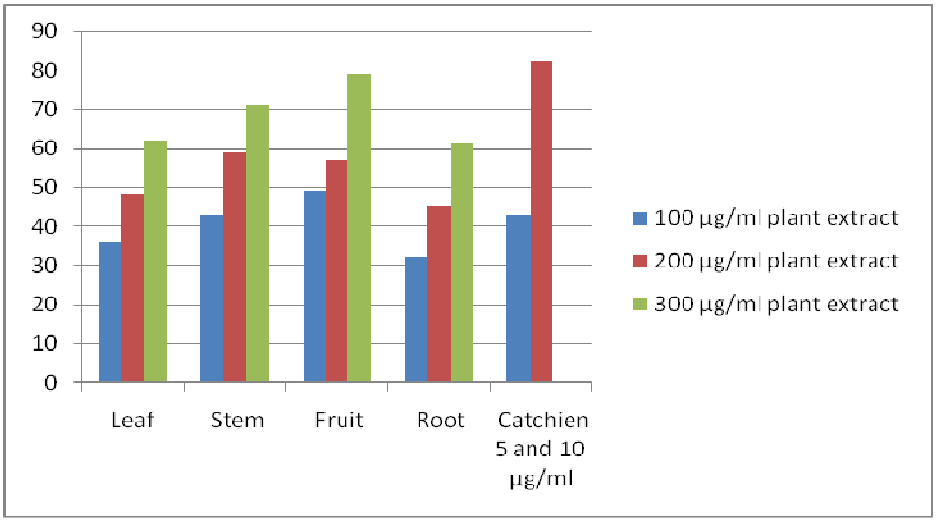

Figure 2

Yield percentage of total phenolic content (100 g) of various parts of Physalis angulata at various concentrations compared to reference standard tannic acid

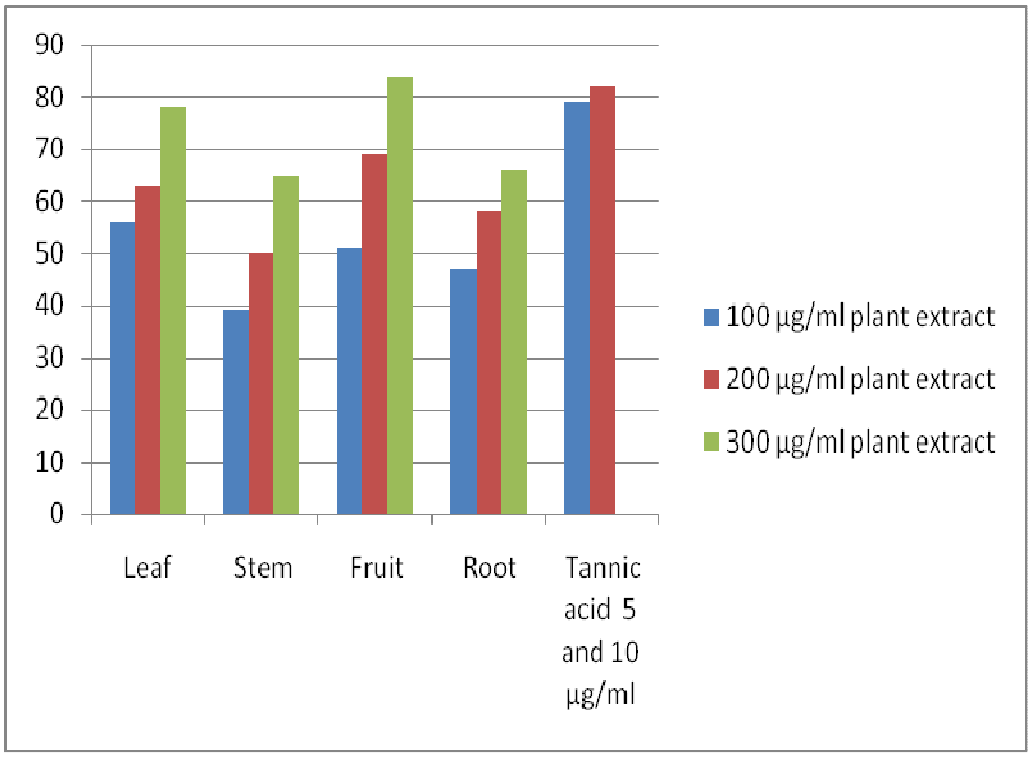

Table 1
Antioxidant activity of Physalis angulata various parts at different concentrations

	DPPH			Super Oxide			Nitric Oxide			H_2O_2			Hydroxyl Radical		
	#100	#200	#300	#100	#200	#300	#100	#200	#300	#100	#200	#300	#100	#200	#300
Leaves	0.2 ± 0.11*	0.35 ± 0.20*	0.25 ± 0.14*	0.45 ± 0.26*	0.25 ± 0.14*	0.3 ± 0.17*	0.25 ± 0.14*	0.34 ± 0.2*	0.17 ± 0.1*	0.28 ± 0.16*	0.45 ± 0.26*	0.30 ± 0.17*	0.20 ± 0.12*	0.05 ± 0.03*	0.36 ± 0.20*
Stem	0.1 ± 0.08*	0.3 ± 0.2*	0.1 ± 0.06*	0.37 ± 0.21	0.51 ± 0.2*	0.47 ± 0.27*	0.20 ± 0.12*	0.28 ± 0.16*	0.32 ± 0.18*	0.15 ± 0.08*	0.1 ± 0.05*	0.43 ± 0.25*	0.47 ± 0.27*	0.51 ± 0.29*	0.56 ± 0.32*
Fruits	0.41 ± 0.24*	0.2 ± 0.1*	0.43 ± 0.25*	0.4 ± 0.23	0.15 ± 0.08*	0.36 ± 0.20*	0.1 ± 0.05*	0.45 ± 0.26*	0.50 ± 0.29*	0.17 ± 0.1*	0.37 ± 0.21*	0.11 ± 0.06*	0.20 ± 0.12*	0.23 ± 0.13*	0.34 ± 0.2*
Roots	0.40 ± 0.23*	0.1 ± 0.05*	0.49 ± 0.28*	0.51 ± 0.29*	0.1 ± 0.05*	0.2 ± 0.11*	0.26 ± 0.15*	0.23 ± 0.11*	0.35 ± 0.20*	0.05 ± 0.03*	0.20 ± 0.12*	0.1* ± 0.005*	0.41 ± 0.24*	0.52 ± 0.30*	0.55 ± 0.31*

Values are mean±S.E.M. n=3
****significance is set at p>0.5.***
 # Conc µg/ml

CONCLUSION

Upon the results produced in the present studies, it is concluded that the methanolic fruit extract of *Physcalis angulata* L. reported to possesses significant anti-oxidant activity. Based on this investigation reports it is suggests that the fruit of this plant is a immense source of natural antioxidants and could play a important role as therapeutic agents to prevent oxidative stress and related disorders.

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