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Antioxidant Potential of *Solanumkurzii* Br. Berry: A Folk Medicinal Food Berryused in Arunachal Pradesh, North-East India

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I. Introduction

Nature has endowed plant kingdom with full of resources. Plant kingdom basically produced two types of compounds; plants nutrients to function directly for primary metabolic processes to regulate growth development and reproduction and allelochemicals or plant secondary compounds as plant chemical defences[1]. Since antiquity the plant resources has been used by human being for food and medicine. Let food be your medicine, once said Hippocrates over 2500 years ago.The civilizations in West assiduously follow the aphorisms of Hippocrates, "*Let food be thy friend and enemy*" for more than 200 years[2].In the second century before Christ, Marcus Porcinus Cato- the well-known Roman senator, lawyer and the enemy of Carthage used cabbage as food and a curative; he even tried to cure his ill wife and son with cabbage[2]. When a food becomes drugs; it is termed as "medical foods". The links of diet and health are no longer questioned[1]. Food and medicine represent a continuum rather than artificial categories; Overlapping nature of traditional food system and medicine lead to the investigation of phytochemicals that explains the food culture and health outcomes[3]. Any of the edible wild plants that are included in local food baskets have both therapeutic and dietary functions and such medicinal foods have been part of Eastern Medicinal theories since ancient times and have recently received attention in the USA and Europe within the fields of functional foods, nutraceuticals and phyto-nutrients[4]. Nutritional therapies including the use of alternative traditional medicinal plants and herbal food with various principles and properties have witnessed renewed interest in the last few decades[5, 6, 7].The knowledge on plant as medicine is orally transferred from generation to generation as "traditional medicines". Traditional medicines are still practices in many pockets of tribal belts all over the world. The role and importance has been identified by World Health Organization and figured at around 80% in developing countries those who depends traditional medicines in primary health care system.Fruit and vegetables are major sources of dietary antioxidant[8]. Dietary antioxidants prevent oxidative damages. Antioxidant of a plant is largely contributed by presence of phenolic compounds and flavonoids[9]. Large numbers of wild edible plants are rich in phenolic compounds[10, 11]. Crude extracts of herbs and spices, therefore plant materials rich in phenolic compounds are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritive value of food[12].

In India, 461 ethnic groups are recognized as Scheduled Tribes. These are considered to be India's indigenous peoples; the largest concentrations of indigenous peoples are found in the seven states of North-East India, including Arunachal Pradesh and the so-called "central tribal belt". Arunachal Pradesh, an Indian state lies in North East of India, is a home of 26 major tribal people and more than 110 sub-tribes. The traditional practices, festive celebrations and traditional knowledge is as rich as the tribes itself, geographical isolation from the Indian mainland has brought them certain distinctive characteristics in culture and customs, the state has many dimensions in food habits and flavour. Indigenous people use numerous herbs, fruits, animals, insects, worm etc. in their folk food[13].

The indigenous people of East Siang District of Arunachal Pradesh, Indiause Solamunkurzii(Fig. 1) as folk food as well as folk medicine. Berry is eaten raw with locally prepared black alcoholic drink called

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"apong" in Adi or boiled and mashed with chilly and dried bamboo shoot powder (fig. 3) either berry is smoke dried over the fire and powdered with salt and chilly (fig. 4); berry powder is preserved in bamboo culmover fire place in a shelf locally called "boring or perap" for long period storage. Fresh berry (Fig.2) is advised to chew in toothache; and also used as expectorant during cough and cold, the water extract of berry is given to patient of stone problem. Berry is also used as appetizer and roughage. Solanumkurzii Br. (Solanaceae) is a shrub, grows naturally in burn and slash cyclic"jhum field" and domesticated in home-garden too, The Adi tribe locally calledSolanumkurzii as "kopir". Upto 4 ft. high with densely stellate-tomentose leaves, flowers purplish in densely stellate woolly racemes, berry glabrous, globose, bitter and orange on ripe[14].

In recent past, wild fruit and antioxidant potential from North East India, in particular; wild fruit found in Arunachal Pradesh have been studied by Jambey et al., (2012)[15] on Garcinia species fruit and Payum et al.,(2013)[13] on Phoebe cooperiana fruit. Literature achieve has no recordable record to be recorded on Solanumkurzii berry in relation to phenolic, flavonoid and antioxidant activities work till date. Present study was carried out to determine total phenolic content, total flavonoid content and antioxidant potential of ethno-biologically and culturally useful folk medicinal food berry of Solanumkurzii among the indigenous people of Arunachal Pradesh, North east India.




Figure 1 : S.kurzii(Twig). *Figure 2 :* Berry *Figure 3 :* Boiled berry *Figure 4 :* Bowdered berry

II. Material and Methods

Chemicals and Solvents : The chemicals 2,2-Diphenyl-1-picrylhydazyl (DPPH), Gallic acid, ferric chloride, 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were obtained from Sigma-Aldrich (Munich, Germany). Merck's Folin-Ceocalteu was used and other reagents and chemicals of analytical grade were Merck (Mumbai, India) and RANKEM (New Delhi, India).

Preparation of crude extract : Fresh berry were collected from Renging Village, Mirem Village, Sile Village and Napit Village of East Siang District, Arunachal Pradesh, India. Berries were cleaned with distilled water before oven dried at 55 degree Celsius and heated till constant weight was achieved; dried berries were grinded in laboratory mill and kept in air tight container for future use. 100g powder were soaked in 500 ml methanol for 48 hrs and filtered through Whatman paper No.41. The residue was re-extracted twice with 500 ml of methanol each. The total filtrate was concentrated by rotatory evaporator at 45^0 C under reduced pressure and stored at -40^0C until analysed.

Determination of Antioxidant Activity using 2, 2-Diphenyl-1-picrylhydazyl (DPPH) Free Radical Scavenging Method: DPPH stable free radical method is an easy; rapid and sensitive way to survey the antioxidant activity of specific compound or plant extracts. The antioxidant activity was determined according to the method of Aoshima et al., [16]. Briefly, to 100 μl of sample extract, or standard, 2.9 mL of DPPH reagent (0.1mM in methanol) was added and mixed vigorously. The reaction mixture was stored in the dark for 30 minute at room temperature and decolouration of DPPH was measured against a blank at 517 nm using an ultraviolet-visible (UV-Vis) spectrophotometer (Lamda-25, Perkin Elmer, Cambridge UK). Linear calibration curves were produced with R^2=0.9998 *(Fig. 5.)* and result was calculated as Trolox equivalent per *gram dry sample.* The inhibition % was calculated using the formula:

$$\text{Inhibition\%} = \frac{\text{A (control)} - \text{A (test sample)}}{\text{A (control)}} \times 100$$

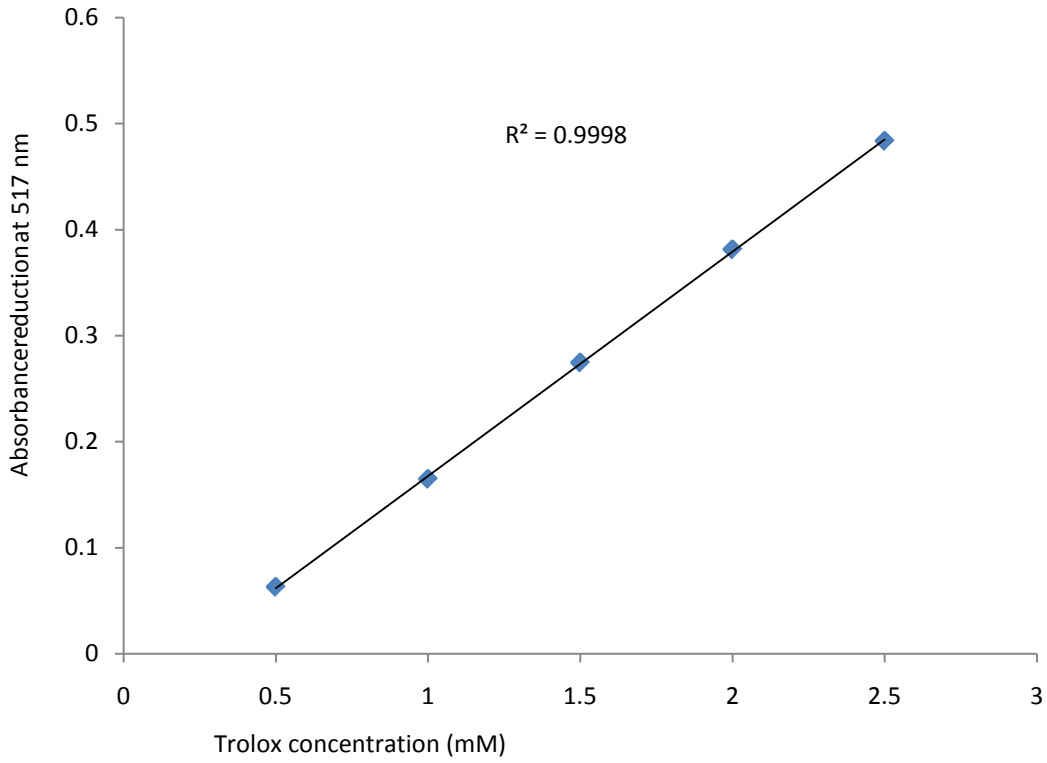

Figure 5 : Trolox concentration vs absorbance of DPPH standard curve

ABTS Free Radical Scavenging Assa : The ABTS radical cation scavenging activity was performed according to Re et al., [17]with slight modifications. The ABTS solution (7mM) was reacted with potassium persulfate (2.45mM) solution and kept overnight in dark to yield a dark green-colour solution containing ABTS radical cation. Prior to use in the assay, the ABTS radical cation was diluted with 50% methanol for an initial absorbance of about 0.700± 0.02 at 734nm using UV-Vis spectrophotometer with the temperature set at 30 ^{0}C. Free radical scavenging activity was assayed by mixing 100μL of test sample with 2.9ml of an ABTS working standard in a microcuvette. The decrease in absorbance was measured at exactly 1 minute after mixing the solution and then at 1 minute intervals up to 6 minutes when final absorbance was recorded. Linear calibration curves were produced with R^2= 0.9986 ***(Fig. 6.)*** for evaluation of antioxidant activity in ABTS and result was calculated as Trolox equivalent per gram dry sample. The inhibition % was calculated using the formula:

$$\text{Inhibition\%} = \frac{\text{A (control)} - \text{A (test sample)}}{\text{A (control)}} \times 100$$

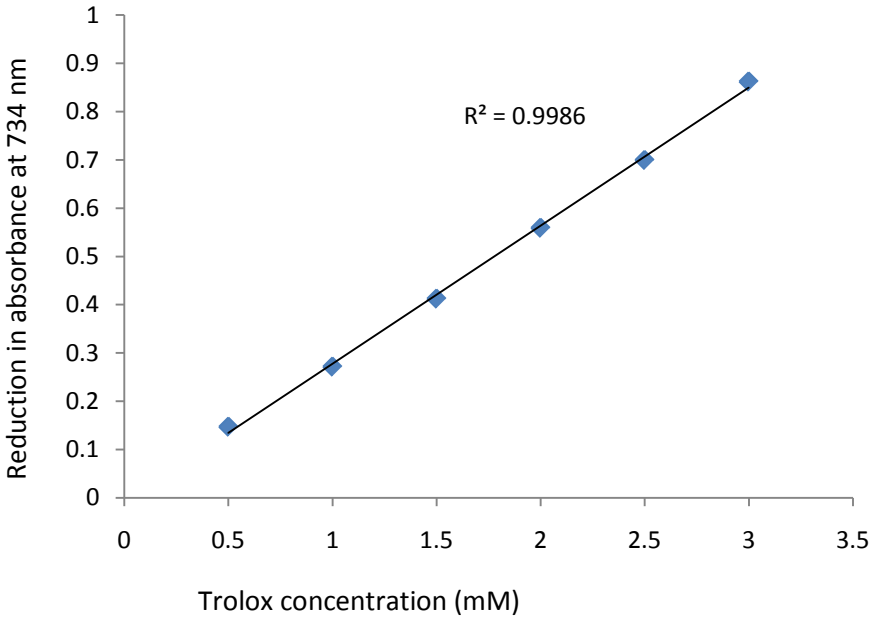

Figure 6 : Trolox concentration vs absorbance for ABTS standard curve

Determination of Total Phenolic Content : Total phenolic content was determined by the Folin-Ciocalteu method[18].Briefly, to 900μL of distilled water and 1mL of the Folin-Ciocalteu reagent 100μL of filtered extract was added. After 5 minutes, 2mLof saturated sodium carbonate (75g.L-1) and 2 mL water was added. Absorbance of the resulting blue- colored solution was measured at 765nm using UV-Vis spectrophotometer after incubation at 30 0C for 1.5 h with intermittent shaking. Quantification measurement was performed based on a standard calibration curve of 20, 40, 60, 80 and 100mg/100mL of Gallic acid in 80% methanol. Total phenolic content was expressed as Gallic acid equivalent (GAE) in the dry sample. Linear calibration curves were produced with R^2=0.9989 (***Fig.7).***

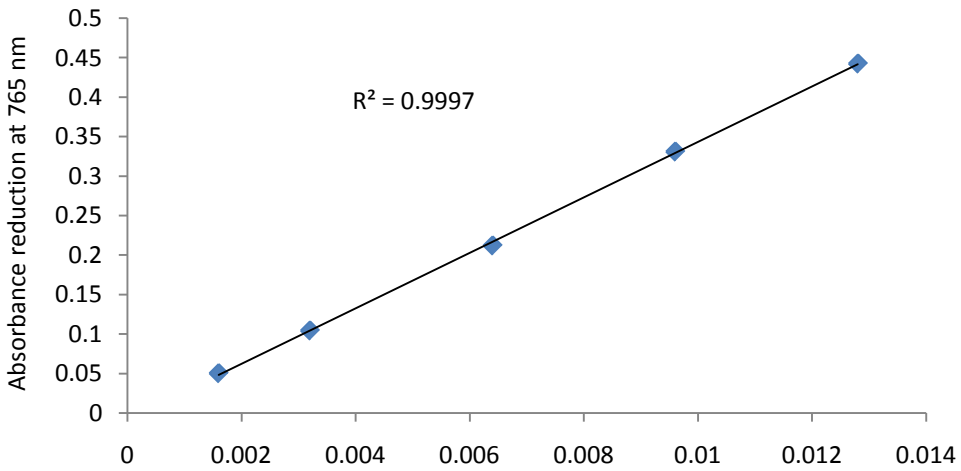

Figure 7 : Gallic acid standard curve for TPC

Determination of Total Flavonoid Content : Total flavonoid content was determined by using the colorimetric method of Sahreen and khan[19] with slight modification. 50mg of sample was dissolved in10 ml of 80% aqueous methanol and filtered through Whatman filter paperNo.42 (125mm). In a 10mL test tube, 0.3ml of extract, 3.4 mL of 30% methanol, 0.15 mL of 0.5M sodium nitrite, and 0.15 mL of 0.3 M aluminium chloride hexahydrate were added and mixed. After 5 minutes, 1mL of 1M sodium hydroxide was added. The absorbance of the mixture was measured at 510 nm using UV-Vis spectrophotometer (Lamda-25, Perkin Elmer Cambridge, UK) and values were express as Rutin equivalent antioxidant capacity. Linear calibration curves were produced with R^2=0.9996 **(Fig.8)**.

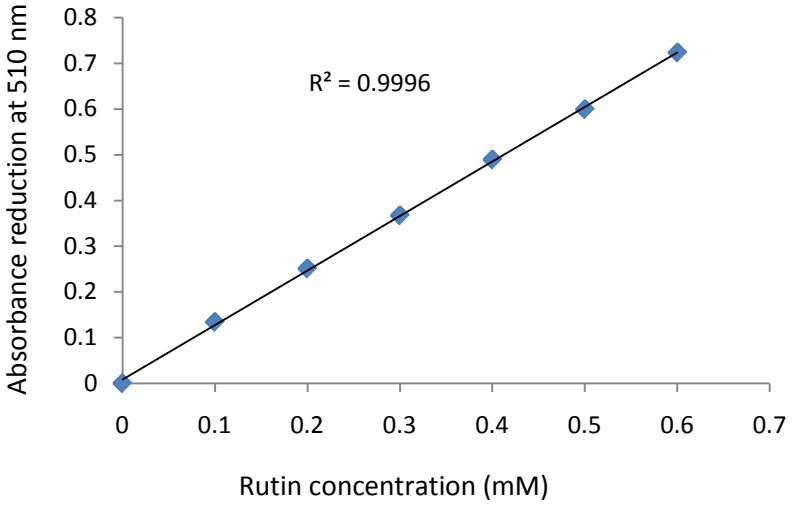

Figure 8 : Rutin standard curve for flavonoid content

Statistical Analysis : All the assays were carried out in triplicate and the experimental results obtained were expressed as mean SD.

III. Results and Discussion

DPPH Assay : DPPH assay is one of the methods used to determine the antioxidant potential of plant extract[20]. DPPH method is based on decrease in purple/dark violet colour of alcoholic DPPH solution[21, 22] when contracted with antioxidant substances like phenolic compounds and have a strong absorption range at 517 nm [23]. The DPPH Assay of *Solanumkurzii* berry is calculated to 257.74 μM/g±2.14.

ABTS : The pre-formed radical cation of 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS*+) is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants[17] , the ABTS assay of the shoot of *Solanumkurzii* was evaluated to 30.75 ±2.14 μM/g.

Total Phenolic Content (TPC) : Phenolic content of plant act as primary antioxidants or free radical scavenger[21]. Significant correlations have been reported with phenolic content and antioxidant activity[22].TPC measured by FolinCiocalteu's method was calculated by plotting Gallic acid standard curve. TPC in mg GAE/g was found as 14.6±4.25.

Total Flavonoid Content (TFC) : Flavonoids are class of secondary metabolites with significant antioxidant and chelating properties. Antioxidant activity of flavonoid depends on the structure and substitution pattern of hydroxyl groups[23, 24, and 25]. The flavonoid content is determined by method by using Aluminium chloride colorimetric assay, 89.00 μMRE/g±2.31 flavonoid content was found.

Table 1 : TPC, TFC, ABTS and DPPH values of the methanolic extract of the *Solanumkurzii* berry

Sample	TPC(mg GAE/g)	TFC (μMRE/g)	ABTS (μM/g)	DPPH (μM/g)
*Solanumkurzii*berry	14.60	89.00	30.70	257.74

IV. Conclusion

The Solanumkurziiberry is highly consumed folk medicinal food among the indigenous people of Arunachal Pradesh. The seedling is seen to grow naturally when jungle is clear and burnt for jhum cultivation, the germination is expected to related with the burnt soil or need high temperature as the plant is not commonly seen grow wild either. The need of further scientific investigation of the plant on germination

,pharmacognosy, phytochemical and proximate is felt. The berry contains considerable phenolic and flavonoid compounds with considerable antioxidants activities.

V. Acknowledgements

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