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Antioxidant Potential of *Solanum Spirale* Shoot and Berry: a Medicinal Food Plant Used in Arunachal Pradesh

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ABSTARCT

Solanum spirale is a tribal medicinal food plant which is used among the Adi tribes of Arunachal Pradesh, India. The methanol extract of shoot and berry were studied for their total phenolic (Folin-Ciocalteu's method) and total flavonoid contents (colorimetric method) to study antioxidant potential ((DPPH & ABTS). The extracts were recorded to contain considerable amounts of phenolic content (18.64±2.12 mg GAE/g) in shoot and 11.72±1.42 mg GAE/g) in mature berry and flavonoid content of (5.29±1.01 µM RE/g) in shoot&50.52±1.03 µM RE/g) in mature berry and antioxidant potentials were calculated as 29.85±1.25 µM/g in the ABTS assay of shoot and38.27±1.33µM/g in the ABTS assay of berry while 45.4±0.37µM/g in the DPPH assay of shoot and 52±0.92 µM/g in the DPPH assay of mature berry respectively. This work also discourse traditional use of *Solanum spirale* among Adi tribes of Arunachal Pradesh.

Keywords: *Solanum spirale*, total phenolic, flavonoid content, antioxidant potential, tribals, medicinal food, ethnobotany.

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INTRODUCTION

Let food be your medicine, once said Hippocrates (c. 460 – c. 370 BC) over 2500 years ago [1, 2]. Galen- "the father of observational medicine" believed that the fundamentals of good medicine lay in diet; for him "diet saves life- surgery takes it away"[3]. Such medicinal food concepts and belief is still observable in the indigenous food systems practices among indigenous people in various pockets of the world, particularly in Arunachal Pradesh[3]. In the word of Etkin & Ross [4] wild plants that are retained in local food cultures are inseparable from traditional therapeutic systems. To Guarrera&Savo[1] also, there is no clear dividing line between food and medicinal plants especially in indigenous and local traditions; Food and medicine represent a continuum rather than artificial categories typically imposed in western medicine. Nature has endowed the North East India with full of wild edible vegetables and fruits and Indigenous people of this region use these resources in their diet as a culture. Food without dal, wheat, potato, oil and spice are the main characteristic features of the indigenous food system in Arunachal Pradesh, it is an interesting researchable domain [3]. Locality use locally available wild and semi domesticated herbs in their diet in Arunachal Pradesh, which are mostly composed of leafy, fruit, tuber and stem vegetables. Fruit and vegetables are major sources of dietary antioxidant[5]. Antioxidant of a plant is largely contributed by presence of phenolic compounds and flavonoids[6]; wild edible plants are rich in phenolic compounds[7]. *Solanum spirale* Roxb.is an undershrub up to 12 ft. high plant; stem erect with 1 or 2 sharp ridges. Leaves are 2-7 by .8-3 inch, (each leaf is subtended by a small leaf often much reduced.), elliptic, entire, acute, membranous, glabrous; lateral nerves about 7 on either half; base alternate; petiole .6 in. long; Flowers are white, small and dense, spirally arranged, racemose, extra axillary, inflorescence. Ripe berries are orange-red in colour, globose, .3 inch in diameter[8]. Aditibes of Arunachal Pradesh use *Solanum spirale* as food and medicines too[9].

MATERIALS AND METHOD

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) [10].

Preparation of crude extract:

Fresh shoot and berry were collected from Balek Village of East Siang District, Arunachal Pradesh, India. Shoot and berries were cleaned with distilled water before oven dried at 40 degree

Celsius and heated till constant weights were achieved; dried shoot and berries were grinded in laboratory mill separately and kept in air tight containers. 100g powder each were soaked in 500 ml methanol for 48 hrs and filtered through Whatman paper No.41. The residues were re-extracted twice with 500 ml of methanol each. The total filtrates were concentrated by rotatory evaporator at 45^0 C under reduced pressure [10].

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., [11]. 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 (Figure 1) and result was calculated as Trolox equivalent per gram dry sample. The inhibition % was calculated using the formula[10]:

Inhibition%= A (control)-A (test sample) / A (control) X 100

ABTS Free Radical Scavenging Assay:

The ABTS radical cation scavenging activity was performed according to Re *et al.,* [12] 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 (Figure 2) 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[10]:

Inhibition%= A (control)-A (test sample)/ A (control)X 100

Determination of Total Phenolic Content:

Total phenolic content was determined by the Folin-Ciocalteu method[13]. 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(Figure 3)[10].

Determination of Total Flavonoid Content:

Total flavonoid content was determined by using the colorimetric method of Sahreen and Khan[14] 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 (Figure 4)[10].

Statistical Analysis:

All the assays were carried out in triplicate & the experimental results obtained were expressed as mean SD.

RESULTS AND DISCUSSION

Table 1: TPC, TFC. ABTS and DPPH values

Sample	TPC(mgGAE/g)	TFC(µMRE/g)	ABTS(µM/g)	DPPH(µM/g)
Solanumspirale(shoot)	18.64±2.12	5.29±1.01	29.85±1.25	45.4±0.37
Solanumspirale(mature berry)	11.72±1.42	50.52±1.03	38.27±1.33	52±0.92

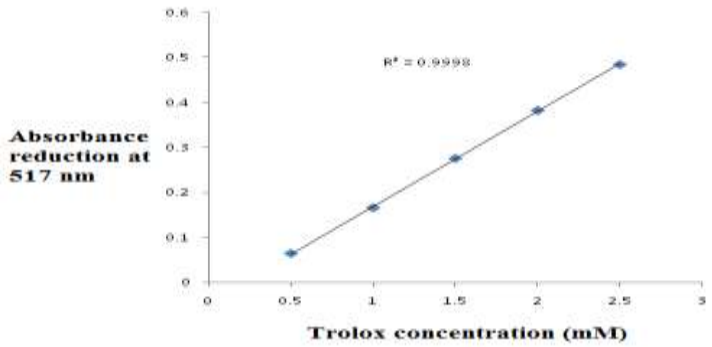

Figure 1: Trolox concentration vs absorbance of DPPH standard curve

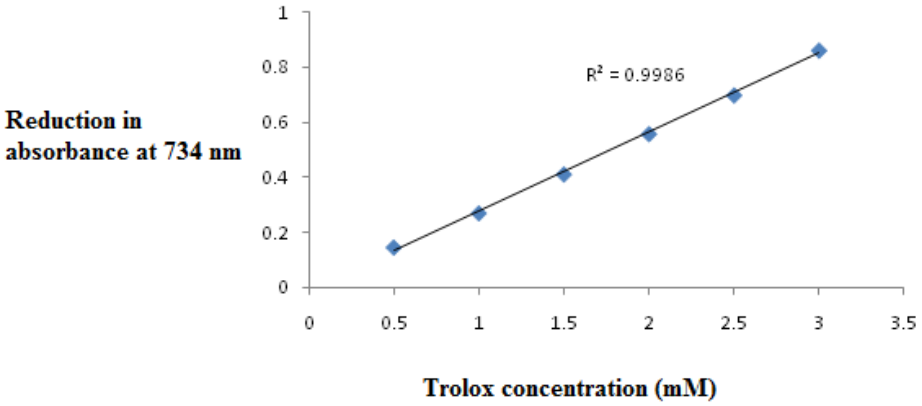

Figure.2: Trolox concentration vs absorbance for ABTS standard curve

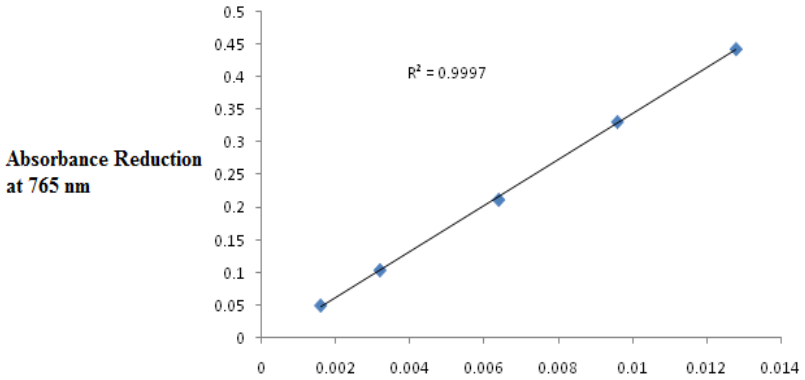

Figure 3: Gallic acid standard curve for TPC

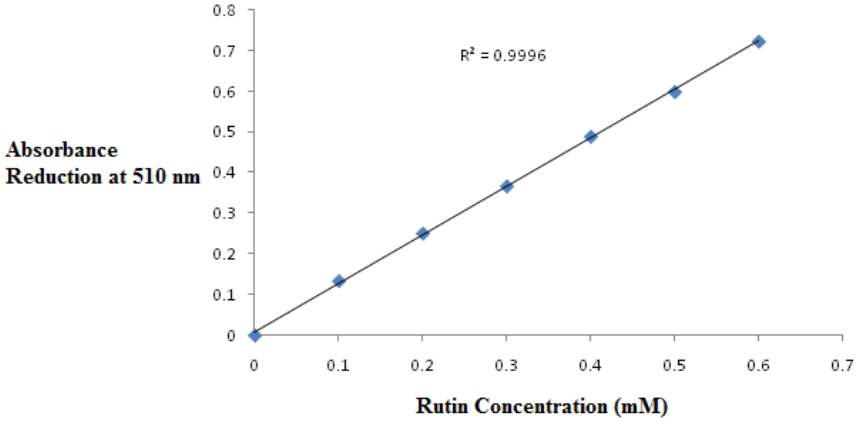

Figure 4: Rutin standard curve for flavonoid content

All forms of aerobic life are constantly subjected to oxidative pressure from reactive oxygen species (ROS), produced during the biochemical utilization of O_2[15]and under stress, our bodies produce more reactive oxygen species[16]. When excess in ROS is produced in body it perturb the redox balance and produce a variety of changes though lipid peroxidation and protein and nucleic acid damage eventually be responsible for cancer, cardiovascular diseases, ageing, and

neurodegenerative disorders[17]. One of the solutions to this problem is to supplement the diet with antioxidant compounds that are contained in natural plant sources[18]. These natural plant antioxidants rich in phenolic and flavonoids as seen in *Solanum spirale* plant can therefore serve as a type of preventive medicine. In the present work total phenolic and flavonoids is taken as the base study to correlate antioxidant potential of the selected medicinal food plants by using free radical scavenging methods of DPPH and ABTS in which Gallic acid, Trolox and Rutin were used as standards. In this study it was recorded that *S. spirale* leaf contain18.64 mg Gallic acid equivalent per gram total phenolic, 5.29µMRE/g total flavonoid content with scavenging activity of 29.85µM/g in ABTS method and 45.4 µM/g scavenging in DPPH method. *S. spirale* berry is recorded to contain 11.72 mg total phenolic content calculated in Gallic acid equivalent per gram, 50.52µM per gram total flavonoid content calculated in equivalent to Rutin with scavenging capacity of 38.27µM/g in ABTS method and scavenging capacity of 52 µM per gram in DPPH method. The considerable antioxidant potential of this berry may be attributed to the flavonoid and phenolic compounds; further, correlation in the total content of flavonoid and DDPH scavenging activities is observed in the *S. spirale* berry.

Figure 5: *S. spirale*

Figure 6:*S. spirale* twig

CONCLUSION

The shoot and berries of *Solanum spirale* are edible. Adi tribes of Arunachal Pradesh in India use shoot and berries as food as well as medicines; These parts also contains adequate phenolic and flavonoids; the antioxidant potential as calculated may be attributed to the total phenolic and flavonoids contents in this medicinal food plant.

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