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Phytochemical Screening and Antioxidant activity of aqueous extract Of *Allophylus serratus* fruit.

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Abstract: The present work aimed to determine the quantitative screening of phytochemicals and Antioxidant activity of *Allophylus serratus* fruit aqueous extract (A.S.Fr.). Results showed the presence of phenolic compounds, flavonoids, alkaloids, phenols and proteins in the extract and showed antioxidant capacity with high amounts of Total phenolic content (63.60 ± 0.88 mg $GAEg^{-1}$), Total flavonoid content (44.51 ± 0.46 mg QEg^{-1}). The radical scavenging activity measurement, done by DPPH assay (EC_{50} = 29.24 ± 0.18 µg/mL) and ABTS assay (EC_{50} = 21.64 ± 1.35 µg/mL) revealed that, fruits of *Allophylus serratus* found to be potential source of natural antioxidants and therefore, recommended for utilization as natural nutritional supplemented medicine which supported the traditional claim by herbal healers.

Keywords: *Allophylus serratus,* Aqueous extract, Antioxidant Activity,

Introduction

Phytochemical processing of raw plant material is essentially requisite to optimize the concentration of known constituents and also to maintain their activities. Recently, interest has noticeably amplified in finding naturally occuring antioxidant to replace synthetic antioxidants (Benabdallah *et al*., 2016). Nutraceutical and herbal medicine prominence is typically allied to their antioxidant power, which can be determined by spectrophotometric methods that can be influenced by phytocompounds which demonstrate antioxidant behavior (Oliveira Neto *et al*., 2017; Leite *et al*., 2018).

Awareness of medicinal plant usage is a result of the many years of struggle against diseases and man learned to pursue drugs in barks, seeds, fruits, and other parts of the plants (Srivastava, 2018). By combining the knowledge derived from traditional medicinal practices with modern science, the possibilities for drug discovery and use of plants in the treatment of wide array of conditions seems endless (Reid *et al*., 2018). In a way to find the substitute of synthetic anti-oxidant drugs and after perusal of literature and by enquiring traditional therapists, Village vaidyas, Ayurvedic physicians, it was found that some of the local plants were used as medicine in different dosage, with admixtures of other material for protection against oxidative stress, body abnormalities, Lithergic conditions, Indigestion, constipation etc., without knowing the hidden biomolecules which act inside the body. It is interesting to document and reveal the bio constituents and its antioxidant potency in the naturally available sources.

In our recent Medicobotanical studies conducted during (2016-2018) around Sri Kalahasti area of Chittoor dt. in Rayalaseema region the Natu-Vaidhyas, Village herbalists, Traditional herbal

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healers claimed that *Allophylus serratus* fruits in decoction form were prescribed as a multifaceted drug and prominent natural remedy for strength adding gastric ailments, stress related, against heat and for other digestion complication. For the determination of this claim we here in this research, fruits of *Allophylus serratus* of Sapindaceae family for its biochemical analysis and antioxidant potentiality.

Materials and Methods

Collection and identification of plant material

Identification of the *Allophylus serratus* was done by Dr.K.Madhava chetty, Plant Taxonomist, Sri Venkateswara University, Tirupati and Voucher specimen deposition (SVUTY/ ARD/ 3744) was done in the Herbarium, Department of Botany, Sri Venkateswara University, Tirupati. The fruiting stage of *A. serratus* is shown in Figure 1.

Seeds and skins of the mature fruits of *A.serratus* were removed, and the pulps were dried in a convection oven at 50°C for one week.

Figure 1 : **A.** Habitat of *Allophylus serratus*

Figure 1 : **B**. Mature fruits of *A.serratus*

A.serratus pulps (2 kg approx.,) were extracted with 95% EtOH at 60°C for 4 h, and the extract was evaporated under reduced pressure to yield a dark brown residue (55g). The residue was suspended in water were carried out to evaluate the Phytochemicals and antioxidant potency.

Preliminary Phytochemical Analysis

The fruit extract was subjected to qualitative phytochemical analysis for determination of major biochemical ingredients using standard methods (Harborne 1984; Stahl 1989; Raaman, 2006) with slight assay modifications followed by Mahendranath *et al*., (2013 & 2014).

Estimation of flavonols (TFLC)

Total flavonols was appraised using Aluminum chloride assay as described by (Manipal *et al*., 2017). The absorbance of triplicate mixtures was read at 440 nm and results were expressed as equivalents of Quercetin in mg QE/g dry weight.

Estimation of proanthocyanidins (Tp)

The value of proanthocyanidin was estimated based on the procedure described by Unuofin *et al*., (2017) with a minor modification.

Condensed tannins (CT)

Total tannins were determined according to Rebaya *et al.* (2015).

Protein content

Estimation of Protein content was determined by Horowitz (2000) and followed according to Aliakbarkhani *et al*., (2018).

Total phenolic content (TPC)

Quantification of phenolic compounds determined according to the method of Folin-Ciocalteu (FC) (Singleton et al., 1999; Yorulmaz and Konuskan, 2017) with slight modification. Briefly, 5gm of crude extract diluted at 1:20 with methanol : water (6:4) was mixed with 1.2 mL of 7.5% Na_2CO_3 and 1.5 mL of Folin–Ciocalteu reagent diluted in the ratio of 1:10. After incubi-

tion in the dark at 25°C for 2 h, the absorbance of the reaction mixture was measured at 760 nm against a methanol blank using a UV-VIS Spectrophotometer (Hitachi U-1900, Japan). All measurements were made in triplicateS. Gallic acid (0–1000 mg/L) was used as a standard to derive the calibration curve. The phenol contents were expressed in terms of milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW) (Ali *et al*., 2014; Limmongkon *et al*., 2017).

Total flavonoid content (TFC)

Total flavonoid contents were measured according to the Aluminum chloride ($Alcl_3$) colorimetric method (Chang *et al.,* 2002). Total flavonoid contents were expressed as mg catechin equivalents per gram (mgCA/g) through the calibration curve with catechin. The calibration curve range was 50–400 mg/mL. The calibration curve was made by preparing quercetin solutions at different concentrations.

Antioxidant Activity

The antioxidant power of the crude extract of *A.serratus* pulps were evaluated by DPPH, ABTS, and EC_{50} values are the effective concentration in which DPPH and ABTS radicals were scavenged by 50% and are often used to evaluate the antioxidant power (Brand-Williams *et al.,* 1995; Shimada *et al.,* (1992); Oliveira-Neto *et al*., (2017). EC_{50} values of the extract, fractions and BHT tested at the same condition are listed in Table 1.

The antioxidant activity was determined according the equation mentioned below:

$$\textbf{Antioxidant activity}\ (\%) = (A_{control} - A_{sample}/A_{control}) \times 100$$

where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the tested sample. The concentration of extract that could scavenge 50% of the DPPH radicals (IC_{50}) was calculated. Trolox and Butylhydroxyl toluene (BHT) were used as positive control.

Results and Discussion

Preliminary phytochemical analysis

The qualitative phytochemical screening of the extract revealed the presence of Alkaloids, Anthocyanins, Flavonoids, Indoles, Steroids, Carbohydrates, Phenols, Proteins, Saponins, Lignins (Table 1).

Table 1. Preliminary Phytochemical screening

S.No.	Phytocompound	Result
1.	Alkaloids	+++
2.	Flavonoids	+++
3.	Indoles, Terepenoids	-
4.	Anthocyanins	++
5.	Steroids	+
6.	Carbohydrates	++
7.	Phenols	+++
8.	Proteins	+++
9.	Saponins	++
10.	Lignins	++

Quantitative analysis of antioxidants

The results for antioxidants of *A.serratus* (mean ± standard deviation) were tabulated (Table : 2).

Table 2. Antioxidant compounds

Variable	Quantity
TPC (mg GAE/gDW)	63.60 ± 0.88
TFC (mg QE/gDW)	44.51 ± 0.46
TFl.C (mg QE/gDW)	24.69 ± 0.24
TP (mg CA/gDW)	26.16 ±1.42
CT (mg RU/gDW)	22.36 ± 1.06

Equivalent's: RU = Rutin, CA = Catechin, GAE = Gallic acid equivalent, QE = Quercetin. The data represent the mean ±SD of triplicate assay for each sample.

Table 3: Estimation of pH, Moisture, Sugar value

Variable	Quantity
pH	6.4 ± 0.8
Protein Content (g 100-1 g)	49.84 ± 2.44
Moisture content (g 100-1 g)	89.62 ± 2.42
Sugar content (g 100 g-1 DW)	72.4 ± 4.02

Table 3 represents estimation of pH, Moisture, Sugar value expressed in g 100 g^{-1} DW of the extract.

The total phenolic content (TPC) of the extract was 63.60 ± 0.88 mg gallic acid equivalent/g dry weight. The total flavonoid content (TFC) of the extract was 44.51 ± 0.46 mg of quercetin equivalent/g dry weight. The tannin content (CT) was 22.36 ± 1.06mg rutin equivalent/g dry weight. The ascorbic acid content of the extract was 19.62 g/g dry weight of extract.

DPPH and ABTS assay (IC_{50} Value)

DPPH and ABTS assay can measure antioxidant capacity of lipophilic and hydrophilic compounds in the same sample which are very simple, inexpensive and usually employed methods for the determination of antioxidant activity and can give reproducible results (Iqbal *et al.,* 2015).

The results were expressed using the term IC_{50}. The lower the IC_{50} is the higher antioxidant capacity (Brand-williams *et al.,* 1995, Noreen *et al.*,2017). The results show that DPPH value (IC_{50}) as 29.24 ± 0.18 (µg/mL) and 21.64 ± 1.35 for ABTS showed lower scavenging ability on DPPH and ABTS radicals when compared to the synthetic antioxidant BHT (IC_{50}= 23 µg/ml). However, for measuring the IC_{50}, reaction between DPPH free radical and antioxidant of *A. serratus* fruit achieved the steady state in 1&1/2 hr.

Other workers Bharat and Krishna (2017) attempted GC-MS analysis of young leaves of *A. serratus*. Chavan and Gaikwad (2016) studied Phytochemicals isolated from different parts of *A.serratus*. Some of the phytochemicals detected in a present study are well known about their bioactivities, fewer are known but little is known about their bioactivities while some are known but there are no reports on their biological activities.

The total antioxidant capacity of fruits of *A.serratus* may be changed via synergistic, additive, or antagonistic interactions among the phytochemical compounds present in the riped fruit. Pulps have provided phytochemical basis of the principal application to better understand the antioxidant power. This study revealed *Allophylus serratus* fruit aqueous extract (A.S.Fr.) contained high amounts of polyphenols, flavonoids and tannins contents and high antioxidant potential for producing specific health promoting antioxidants which could be attractive to the food or pharmaceutical industry. This edible botanic traditional application is proved to be a potent natural remedy.

Conclusions

In conclusion, by preliminary phytochemical and antioxidant activity screening of *Allophylus serratus* fruit correspond to a high source of natural therapeutic bioactive compounds as bonafied by herbals healers and village traditional physicians and was roved by our research. A further study for the analytical characterization of the biochemical profiles in vivo conditions is required to strengthen antioxidant abilities which may be used as antioxidant drugs and in natural therapies.

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