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Research Article

INVESTIGATION ON REGIONAL VARIATION IN TOTAL PHENOLIC, ALKALOID CONTENT AND I*N-VITRO* ANTIOXIDANT ACTIVITY OF *CHROZOPHORA ROTTLERI L.*

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ABSTRACT

In this study we investigated and compared the total phenolic, alkaloid content and *In-vitro* antioxidant activity of aerial part alcoholic extract of *Chrozophora rottleri* collected from three different regions i.e. Marteru (Godavari river region), Tirupathi (southeren zone) and Lam (Krishna river) regions of Andhra Pradesh, India. Quantitative regional variation was observed in total phenolic and alkaloid content in alcoholic aerial part extracts of *Chrozophora rottleri* from three regions. Concentration dependent antioxidant activity was observed for all these extracts and observed regional variation in three regions of extracts for scavenging of Superoxide, Hydroxyl and DPPH radicals. Among three regions, Marteru region aerial part extract of *Chrozophora rottleri* showed good antioxidant, Phenolic and alkaloid content.

Keywords: Alkaloid content, Antioxidant activity, *Chrozophora rottleri*, Regional variation.

INTRODUCTION

Weeds are treated as unwanted material in the crop fields as they are sharing the nutrients, water and other essentials ultimately affecting the main crop and they are taken out by the farmers as a waste. Many Crop weeds are known to possess medicinal values. However, these weeds of medicinal value are not subjected to systematic phyto-pharmacological evaluation. If a systematic scientific approach is adopted, its acceptability enhances and promotes them as herbal drugs. Because herbal products can have differences in their composition depending on the soil where they grow[1] and agro-climatic conditions. The chemotypic and biological activity variation of medicinal value of weed flora at different climatic zones has not been studied much so far.

Chrozophora rottleri belongs to family Euphorbiaceae, known as shadevi in Hindi, erramirapa, guruguchettu and linga mirapa in Telugu grows commonly as weed in wet land like crop fields and in waste lands like along roads, in stream beds. It is herb to under shrub up to 60cm height distributed in India, Mayanmar, Thailand, Andaman Islands and Malaysia[2,3]. The plant provides a blue color dye. In Nepal, juice of the fruit is given in cases of cough and colds. Since last three decades many plant extracts and biological active molecules from plants reported to have medicinal uses. However, not much work has been done to evaluate the medicinal uses of *Chrozophora rottleri.*The present study was aimed to investigate the influence of regional variation on total phenolic, alkaloid content and *in-vitro* antioxidant activity of aerial part extracts of *Chrozophora rottleri* from Marteru (Godavari river region), Tirupathi (Southeren zone) and Lam (Krishna river) regions of Andhra Pradesh, India. Hence, the present investigation provides an opportunity to profile chemical and *in vitro* antioxidant variations due to different climatic zones.

MATERIAL AND METHODS

Drug and Chemicals

Folin-Ciocalteau (FC) reagent, Bromocresol green (BCG),Riboflavin, 2-Deoxy ribose, Nitroblue tetrazolium(NBT) and 2,2-Diphenyl -1-picrylhydrazyl (DPPH) were purchased from Sigma chemicals, USA. All other chemicals used were of analytical grade.

Plant Material and Preparation of extracts

The plant material was collected from three different regions of Andhra Pradesh i.e., Marteru, Tirupathi and Lam. The authenticity of the plant was confirmed by Taxonomist Prof. M.Venkaiah, Department of Botany, Andhra University, and Visakhapatnam. The Voucher specimens were deposited in the herbarium, College of Pharmaceutical Sciences, Andhra University. The freshly collected *Chrozophora rottleri* aerial parts were washed with distilled water and then shade dried at room temperature for 10 days. The shade dried material was pulverized into courses powder and extracted by maceration process by using ethanol (70%v/v) solvent.The extracts was filtered and concentrated to crude extract in a rotary vacuum evaporator (Buchi-R210,Switzerland) at 40°C and used for further investigation.

Quantification of Total Phenols

Total phenolic content was determined using the Folin-Ciocalteau reagent[4]. Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765nm.The intensity of the light absorption at that wave length is proportional to the concentration of phenols. By using standard Gallic acid calibration curve, measure the concentration of phenolic content in Gallic acid total equivalents using unit's mg/gm. (GAE).

Total Alkaloid Content

Total alkaloid content was determined by the Fazel et al., 2008 method[5].The plant extract (1mg/ml) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One ml of this solution was transferred to a separating funnel and then 5 ml of BCG solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All experiments were performed thrice; the results were averaged and reported in the form of Mean ± S.E.M.

In-vitro antioxidant activity

Oxygen is essential for survival however, its univalent reduction generates several
harmful reactive oxygen species (ROS), inevitable to living cells and highly associated with the wide range of pathogenesis such as diabetes, liver damage, inflammation, aging, neurological disorders and cancer[6]. The alcoholic aerial part extract of *C.rottleri* from different regions were screened and compared for following free radical scavenging activity.

Superoxide radical Scavenging activity

Superoxide scavenging activity of the plant extract was determined by McCord & Fridovich, 1969 method[7], which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the $Fe^{2+}/EDTA/H_2O_2$ system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances[8].

DPPH radical Scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca et al., 2003[9]. In DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl–picrylhydrazine.

RESULTS AND DISCUSSION

Total Phenolic Content

The alcoholic aerial part extracts of *Chrozophora rottleri* collected from three different climatic regions of Andhra Pradesh i.e. Marteru, Tirupathi and Lam showed variation in Phenolic content ranging from 197.7±0.4 to 671.2±0.24 mg/gm GAE .Lam region contains more phenolic content compared to other two regions (Table 1).

Total alkaloid content

The determined total alkaloid content in alcoholic aerial part extracts of *Chrozophora rottleri* from Marteru, Tirupathi and Lam regions ranged from 61.16±0.53-131.92±0.25 mg/gm. Among three regions Marteru region showed more alkaloid content (Table1).

Table 1: Regional Variation in Total phenolic and alkaloid content (mg/gm) of aerial part extracts of *Chrozophora rottleri* from Marteru, Tirupathi and Lam regions.

Content	Marteru region	Tirupathi region	Lam region
Total Phenolic content (mg/gm)	197.7±0.4	514.6±0.62	671.2±0.24
Total alkaloid content (mg/gm)	131.92±0.25	91.44±0.86	61.16±0.53

***In-vitro* antioxidant activity**

Superoxide radical scavenging activity

Studying the scavenging activity of plant extracts/compounds on superoxide radical is one of the most important ways of clarifying the mechanism of antioxidant activity. In the present study, the alcoholic aerial part extract of *Chrozophora rottleri* was found to possess concentration dependent scavenging activity on superoxide radicals. *Chrozophora rottleri* from Marteru region showed better inhibition of superoxide radical than Tirupathi and Lam regions.

Based on mean IC_{50} values for Superoxide radical scavenging activity the order of free radical scavenging activity of the plant extracts region wise as follows; Marteru region (60µg) > Tirupathi region (66.54 µg) > Ascorbic acid (80.24µg) > Lam region (150.25µg) (Table 2) .

Table 2: *In-vitro* concentration dependent percentage inhibition of Superoxide radical by alcoholic aerial part extracts of *Chrozophora rottleri* from Marteru, Tirupathi and Lam regions.

Conc (µg/0.1 ml)	Marteru region	Tirupathi region	Lam region	Ascorbic acid
50	48.39±2.3	48.63±2.2	33.19±2.6	43.17±0.75
100	54.82±2.12	53.28±2.42	45.67±1.7	52.41±0.26
250	62.38±2.2	62.37±3.1	58.35±2.34	61.10±0.23
500	68.49±1.52	73.57±2.53	62.37±3.2	75.31±1.33
750	72.35±2.4	76.32±3.45	70.40±2.54	81.52±1.61
1000	76.37±2.32	79.70±2.5	74.63±3.3	86.48±0.55
2000	81.51±2.45	81.40±4.30	75.48±3.5	87.17±1.42
IC_{50} value	60	66.54	150.25	80.24

Hydroxyl radical scavenging activity

Among the reactive oxygen species, the hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism[10]. A single hydroxyl radical can result in formation of many molecules of lipid hydroperoxides in the cell membrane, which may severely, disrupts its function, and lead to cell death.

In this study alcoholic extracts of *Chrozophora rottleri* from three regions was found to possess concentration dependent scavenging activity on hydroxyl radicals. Among the extracts aerial part extract of *Chrozophora rottleri* from Marteru region showed better percentage of inhibition for hydroxyl radical. The 50% Inhibition concentrations (IC_{50} values) for superoxide radical by alcoholic extract of *Chrozophora rottleri* and Ascorbic acid were shown in Table 3.

Table 3: *In-vitro* concentration dependent percentage inhibition of Hydroxyl radical by alcoholic aerial part extracts of *Chrozophora rottleri* from Marteru, Tirupathi and Lam regions.

Conc (µg/0.1 ml)	Marteru region	Tirupathi region	Lam region	Ascorbic acid
50	32.50±2.5	33.46±2.5	26.39±2.54	31.67±1.25
100	48.57±2.53	48.37±2.41	40.54±2.3	40.30±1.23
250	65.77±3.2	60.61±3.1	56.60±2.51	55.61±1.13
500	76.29±2.53	68.26±2.5	60.23±1.54	72.27±2.10
750	79.35±3.4	73.61±3.5	68.45±4.22	81.52±1.61
1000	82.60±4.32	80.31±3.62	73.23±2.3	84.70±1.65
2000	85.66±3.12	84.70±3.14	76.48±5.21	84.85±3.24
IC_{50} value	109.25	110.43	190.32	190.2

Table 4: *In-vitro* concentration dependent percentage inhibition of DPPH radical by alcoholic aerial part extracts of *Chrozophora rottleri* from Marteru, Tirupathi and Lam regions.

Conc (µg/0.1 ml)	Marteru region	Tirupathi region	Lam region	Ascorbic acid
50	39.39±2.42	45.30±2.53	46.36±2.42	45.30±2.15
100	45.76±3.42	52.27 ±3.24	53.18±3.24	75.61±2.14
250	58.33±2.5	62.42±3.6	60.45±2.52	81.82±2.41
500	65.15±3.4	72.58±3.12	75.61±3.24	86.52±2.20
750	73.48±2.51	75.76±2.45	78.48±2.12	88.18±2.11
1000	78.33±4.5	81.67±4.2	83.64±2.54	90.15±2.21
2000	78.64±4.52	82.42±3.42	85.76±1.24	90.45±3.42
IC_{50} value	150	85	80.5	60.24

DPPH radical Scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical with a maximum absorption at 517 mm that can readily undergo reduction by an antioxidant. Because of the ease and convenience of this reaction it now has widespread use in the free radical-scavenging activity assessment[11,12].

Based on mean IC_{50} values for DPPH radical scavenging activity *Chrozophora rottleri* from Lam region showed good activity as compared with two regions (Table 4).

CONCLUSION

In present study we found that clear regional variability in Total phenolic, alkaloid content and free radical scavenging activity against Superoxide, Hydroxyl and DPPH radicals. We found that *Chrozophora rottleri* extract from Lam region showed better Phenolic content but Marteru region showed more alkaloid content. Interestingly, in *in-vtro* antioxidant activity *Chrozophora rottleri* extract from Marteru region showed good free radical scavenging activity against Superoxide and Hydroxyl radicals but Lam region showed better inhibition of DPPH radicals. Investigation on regional variation of biological activities for these extracts is in progress.

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