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CHARACTERIZATION OF FEW MEDICINAL PLANTS FROM SOUTHERN ORISSA FOR THEIR FREE RADICAL SCAVENGING PROPERTY.

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ABSTRACT

Free radicals and reactive oxygen species are generated in the body due to various physiological and biochemical processes. They are responsible for various chronic diseases like cancer, diabetes and oxidative damage of DNA and proteins. There is a huge demand of herbal antioxidant compounds in the market. *Padaeria foetida,Tribulus terrestris and Oxalis corniculata* are used in traditional healthcare system to cure various diseases. During the present investigation the biochemical profiling and *In vitro* free radical scavenging activity of methanolic extract of the selected plants have been studied. Total polyphenol content was found highest in *Paederia foetida*(35.67mg of gallic acid equivalent per gram of the extract) followed by *Tribulus terrestris*(25.67 mg of gallic acid/gram of the extract) and *Oxalis corniculata* extracts .(16.87 mg of gallic acid/gram of the extract). The IC_{50} value of the *Padaeria foetida,Tribulus terrestris and Oxalis corniculata* 21.32 µg/ml,22.87 µg/ml and 32.78 µg/ml respectively.

Key words: Free radicals, reactive oxygen species, Polyphenol content,IC_{50}

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INTRODUCTION

Free radicals and reactive oxygen species are generated in living cells as a result of physiological and biochemical processes. Free radicals are the causative agents for many chronic diseases, such as cancer, diabetes, aging and other degenerative diseases in humans usually due to oxidative damage of proteins, lipids and DNA[1] .Plants are the valuable sources of natural products for maintaining human health, more than 80% of population across the world use traditional medicine including compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. [2]Phenolic compounds have antioxidant, antiapoptosis, antiaging, anticarcinogenic, anti-inflammatory activity these biological actions are due to the reducing capacity of phenols.[3]

Paederia foetida L. commonly known as skunk vine belongs to the family Rubiaceae. In India, *Paederia foetida* is found in the Himalayan ranges up to an altitude of 1800 m from sea level. It is also found in Bihar, Odisha, Bengal and Assam. The major classes of chemical constituent present in this plant are iridoid glycosides, sitosterol, stigmasterol, alkaloids, carbohydrates, protein, amino acid and volatile oil.[4]

Oxalis corniculata is a creeper belongs to the family oxalidaceae having wound healing and cardio relaxant activity [5,6]. The ethanol extract of *Oxalis corniculata* plant having nematotoxic activity. [7] Methanolic and ethanolic extracts of *Oxalis corniculata* has antibacterial activity.[8]*Tribulus terrestris* is a prostrate annual herb of family zygophyllaceae. Its fruit extract has urolithiasis effect.[9] The plant saponins from *Tribulus terrestris* have been studied for its hyperlipidemic, aphrodisiac, antilipidemic activity.[10-12] During the present investigation the phytochemical profiling and free radical scavenging activity of the plant extract was studied.

MATERIALS AND METHODS

Plant collection

Approximately 500g of the plant parts collected from the outskirts of Berhampur. The collected plant were identified and authenticated by the competent authority of the research specialization, Dr. Malay Kumar Mishra, Professor & Head Department of Botany, Berhampur University, Orissa.

Plant sample preparation and extraction

Tribulus terrestris ,*Paederia foetida and Oxalis corniculata* were cleaned, shed dried for a week and finally ground to powder by mechanical grinder. 40 g plant powder placed in a cellulose thimble in an extraction chamber, which was placed on top of a collecting flask beneath a reflux condenser. 200 ml of methanol added to the flask and the set up, heated under reflux at 50^0C. This process carried out for 72 hours. Extract was filtered and resultant filtrate was distilled in Vacuum under reduced pressure following [13].

Screening for phytochemicals

The preliminary phytochemical tests were made as per the standardised protocols and method described by Harborne[14]

Thin layer chromatography

one hundred microgram of each extract in (10µl Methanol) was spotted on to the precasted TLC plates(TLC Silica gel 60 F 254 , Merck USA) and developed in a solvent mixture containing Chloroform :Methanol :Water in 33:40:27 ratio. chromatograms were examined under UV light at 365nm.The position of the bands on the TLC was noted by calculating the retardation factor R_f i.e. the distance the compound travelled divided by the distance the solvent had travelled from the origin.[15]

Determination of total Phenolic and Flavonoid content

Total soluble phenolic compounds in the extracts were determined with the Folin-Ciocalteu reagent as per the method

described by Javanmardi et al[16]. 1ml of (1:10) diluted sample extract in 70% ethanol was added to 5ml of ten times diluted Folin Ciocalteu reagent in water. After 3 min 4ml of Na_2CO_3 (10%) was added and the whole content was incubated at room temperature for 60 min in dark. Absorbance was taken at 765nm. All tests were performed in triplicate; the concentration of total phenolic compounds is described as gallic acid equivalent (GAE) in mg/ g of dry material.

The total flavonoid content was determined according to aluminium chloride colorimetric method of Lin and Tang [17]with minor modification. Aliquots of 0.1 g of samples were, dissolved in 1 ml deionised water and this solution (0.5 ml) was mixed with 1.5 ml of 95% alcohol, 0.1 ml of 10% aluminium chloride hexahydrate ($AlCl_3$), 0.1 ml of 1 M potassium acetate (CH_3COOK), and 2.8 ml of deionised water. After incubation at room temperature for 40 min, the absorbance was measured at 415 nm. Rutin was taken as standard.

Determination of total tannin and beta carotene content

Total tannin content was determined by following method. 1 mL of methanolic sample solution was mixed with 5 mL vanillin solution (0.5%) containing 4% of concentrated hydrochloric acid. After incubation at 30 ^{0}C for 20 min, the absorbance of reaction mixture was measured at 500 nm. [18]

Beta carotene content was estimated by the method of Nagata and Yamashita[19]. The dried methanolic extract (100 mg) was vigorously shaken with 10 ml of acetone: hexane mixture (4:6) for 1min and filtered. The absorbance of the filtrate was measured at 453, 505 and 663 nm. β-Carotene content was calculated according to the equation: β-Carotene (mg/100 ml) = 0.216 (A_{663}) - 0.304 (A_{505}) + 0.452(A_{453}).

Determination of total antioxidant activity

Equal volume of extract and reagent were taken in the tubes containing extract and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95 ^{0}C for 90 min. The mixture was cooled to room temperature and the absorbance of each solution was measured at 695 nm against a blank [20].

Determination of DPPH radical scavenging activity

The assay was done as per the procedure of Parejo etal .[21] 44 mg of extract was dissolved in 1 ml of methanol and it is diluted to obtain three different concentrations of the extract i.e. 550µg/ml, 814µg/ml and 1100 µg/ml. Each concentration of the extract was mixed with 2 ml of 1mM methanolic DPPH solution in cuvettes. The decrease in absorbance was measured at 515 nm after 30 minutes using a (UV-visible Shimazdu 1800) Spectrophotometer and the free radical scavenging activity was calculated as below.

Scavenging Activity (%) = [(Acontrol - Asample) / Acontrol]*100.

RESULT &DISCUSSION

Paederia foetida leaf extract is green in colour with characteristic smell and greasy consistency. *Tribulus terrestris* leaf extract is light green in colour with characteristic smell and greasy consistency. *Oxalis corniculata* leaf extract is dark green in colour characteristic smell and sticky in nature. Preliminary phytochemical screening it was found that *Padaeria foetida* extract contains terpenoids, flavonoids, saponins, reducing sugars and alkaloids.*Tribulus terrestris* extract contains flavonoids, saponins, reducing sugar, alkaloids and cardiac glycosides. *Oxalis corniculata* extract contains terpenoids, flavonoids, saponins, reducing sugar, tannins and cardiac glycosides. (Table.1.)

Table.1
Phytochemical composition of Paederia foetida leaf extract, Tribulus terrestris leaf extract and Oxalis corniculata leaf extract.

Types of phytochemicals	*Paederia foetida* extract	*Tribulus terrestris* extract	*Oxalis corniculata* extract
Terpenoids	+	-	+++
Flavonoids	+	++	+++
Saponins	+	+	+
Anthroquinones	-	-	-
Reducing sugar	+	+	+
Tannins	-	-	+
Alakaloids	++	++	++
Cardiac glycosides	-	+	+

+: Present; -: Absent; ++: Moderately positive;+++:Highly positive
Chromatographic profile had shown a red colour spot and a bluish white having Rf value in the range of 0.81 to 0.94 in the solvent system chloroform: methanol: water (33:40:27) at 365 nm UV light. (Table .2.)

Table.2
Detection of phytochemicals in solvent systems Chloroform: Methanol: Water (33:40:27).

S.N. of the spot top→ bottom	Color of the spot at 365 nm UV light	Retention Factor		
		Paederia foetida	*Tribulus terrestris*	*Oxalis corniculata*
1	Red	0.94	-	0.84
2	Bluish white	0.81	0.83	-

Total poly phenol content is highest in case of *Paederia foetida* extract (35.67 mg of gallic acid equivalent/gram of the extract) followed by *Tribulus terrestris* extract(25.67 mg of gallic acid/gram of the extract) and least in *Oxalis corniculata* extract.(16.87 mg of gallic acid/gram of the extract). Betacarotene content is more in case of *Oxalis corniculata* followed by *Paederia foetida* and *Tribulus terrestris*. Tannin content was found in equal concentration in the three extracts. (Table .3.) Total phenolic content found in the range of 16.87 mg of gallic acid equivalent per gram to 35.67 mg of gallic acid equivalent per gram of the extract. Similar kind of studies have been made by various researchers and reported that the range observed was on total phenolic content in different aromatic and medicinal plants from 6.80-32.10 and 4.04-42.09 mg gallic acid equivalents per g dry weight basis. [22-23] As per Margaret etal [24] phenolic content of *Curculigo pilosa* observed 65.17 the significant difference observed when compared these two species of the same genera. The total phenolic content of *Tribulus terrestris* was estimated 14.43 mg gallic acid equivalent per gram dry weight basis, which is slightly less than the findings of the present study with the plant *Tribulus terrestris*. [23]

Total flavonoid content was highest in case of *Oxalis corniculata* followed by *Tribulus terrestris* and *Paederia foetida* extracts. Yuan-Chuen [25]reported flavonoid contents from 3.27 to 4.92 g rutin equivalent /100 g for citrus peels and from 0.07 to 1.62 g catechin equivalents /100 g for Amazonian plants respectively.

Table.3
Total polyphenol, total falvonoid, tannin and beta carotene content of the plant extracts

Extracts	Total polyphenols (mg of Gallic acid equivalent / gram of extract)	Total Flavonoid content (mg of Rutin equivalent / gram of extract)	Beta carotene content (Mg/gram of the extract)	Tannin content (mg of catechin equivalent/gram of the extract)
Paederia foetida	35.67±0.044	0.29±0.005	0.074±0.0380	2.59±0.044
Tribulus terrestris	25.67±0.044	0.45±0.004	0.015±0.0005	2.55±0.044
Oxalis corniculata	16.87±0.044	0.58±0.005	0.175±0.0010	2.60±0.044

The total antioxidant activity of *Oxalis corniculata* extract observed highest (1.44 µg of Ascorbic acid/mg of the extract.) followed by *Tribulus terrestris* and *Paederia foetida* extract.

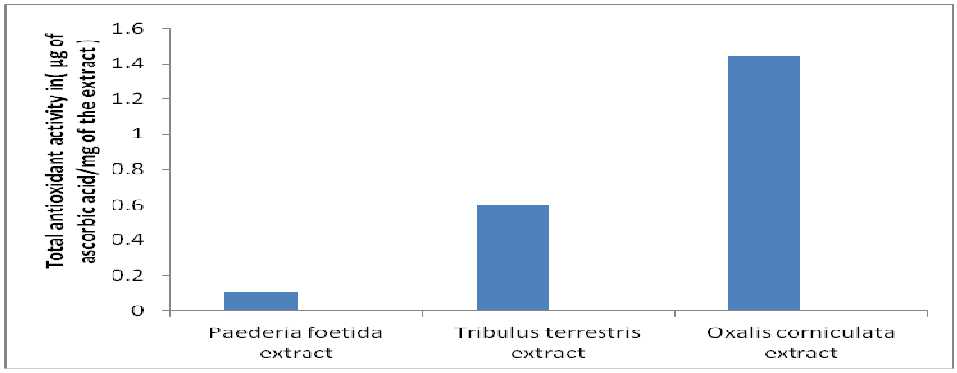

Figure1
Comparision of total antioxidant activity of different plant extracts

The popularity of using the DPPH free radical method for estimating free radical scavenging activity is due to its simple, rapid, economic and relatively more accurate characteristics. The antioxidant activity of individual compounds, present in the extracts depends on structural factors such as the number of phenolic hydroxyl or methoxyl group, flavone hydroxyl, keto groups, free carboxylic group and other structural features. The DPPH radical is considered as the a model of lipophilic radical, a chain reaction in lipophilic radicals was initiated by lipid autoxidation and the radical scavenging activity of plant extract is determined from the reduction in absorbance at 517 nm due to scavenging of stable DPPH free radical. The IC_{50} of *Paederia foetida* leaf extract was 21.32 µg/ml and that of *Oxalis corniculata* and *Tribulus terrestris* is 32.78 and 22.87 µg/ml respectively. The positive DPPH test suggests that the samples are free radical scavengers. Lie-Fen etal [26]described the free radical scavenging activity of 26 medicinal plants and in terms of IC_{50} value that ranged of 4.6 to 187 µg/ml and [27]reported the free radical scavenging activity of *Bauhinia racemosa* L. stem bark and the IC_{50} value was found 152.29 µg/ml. Similar kind of investigations have been made by Elayaraja etal [28] on semi-aquatic, perennial, aromatic herb *Acorus calamus* linn.

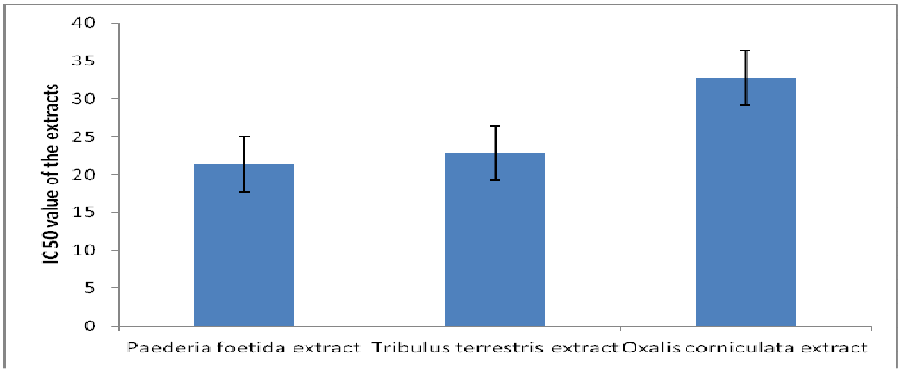

Figure 2
Comparison of IC $_{50}$ value of different plant extracts.

CONCLUSION

During the present investigation its was observed that the IC_{50} value on lower side which indicates the plant extract having better antioxidant activity. All the three plant studied have given significant values, the plant *Paederia foetida* came up as most desired plant for free radical scavenging therefore it can be used as better antioxidant in various forms.

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