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RESEARCH ARTICLE

ANTIOXIDANT ACTIVITY AND POLYPHENOL CONTENT OF SEVEN MEDICINAL PLANTS OF ASCLEPIADACEAE - A COMPARATIVE STUDY

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ABSTRACT

Seven medicinal plants of the family Asclepiadaceae were characterised for their free radical scavenging activity, total polyphenol and flavonoid contents. Antioxidant activity was determined by DPPH (1, 1-diphenyl-2-picryl- hydrazyl) assay method. There was direct correlation between polyphenol content and antioxidant activity (correlation coefficient $R^2 = 0.6557$). IC_{50} value and total flavonoid content were strongly correlated (correlation coefficient $R^2 = 0.8337$). These plants represent promising sources of natural antioxidants and these findings give scientific bases to their ethno pharmacological uses.

INTRODUCTION

Antioxidants can protect the human body from free radicals and ROS (Reactive Oxygen Species) effects [1]. Oxidative damages caused by free radicals to living cells mediate the pathogenesis of many chronic diseases, such as Parkinson's disease, Alzheimer's disease [2], cancers, aging, coronary, heart ailments, cardiovascular diseases [3], atherosclerosis, cataracts and chronic inflammatory diseases, and other degenerative diseases [4]. Medicinal plants contain many antioxidants such as vitamins, carotenoids, flavonoids, polyphenols, saponins, enzymes and minerals [5]. Natural antioxidants tend to be safer and also possess anti-viral, anti-inflammatory, anti-cancer, antimutagenic, anti-tumour, and hepatoprotective properties. The aim of the present study was to examine the total polyphenolic and flavonoid contents as well as the antioxidant activity of leaves of seven medicinal plants of the family Asclepiadaceae.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Asclepias curassavica* L., *Calotropis gigantea* (L.) R.Br., *Gymnema sylvestre* (Retz.) R.Br., *Holostemma ada-kodien* Schult., *Pergularia daemia* (Forsk.) Chiov., *Tylophora indica* (Burm.f.)Merrill., *Wattakaka volubilis* (L.f.) Hasskarl were collected, identified and authenticated. The leaves were separated, removed the dust particles, dried separately under shade, powdered and stored in air tight bottles for further use.

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Estimation of total polyphenols

For the estimation of total polyphenols - 50g of powdered drug extracted 3 times with 100ml methanol. Evaporated the fitrate to dryness. Dissolved the residue in 50ml of distilled water. Extracted aqueous solution with 250 ml of petroleum ether and discarded the ether layer. Extracted aqueous layer with 25ml chloroform and discarded the chloroform layer. Extracted the aqueous layer with 25ml of diethyl ether and discarded diethyl ether solution. Then extracted the aqueous layer with 25ml of ethyl acetate 3 times. Collected the ethyl acetate layer in a previously weighed flask (w_1) after passing through sodium sulphate and evaporated to dryness. Dried the residue at 105°C and weighed (w_2). Percentage of polyphenols = w_2-w_1 x100 /weight of the sample.

Estimation of total flavonoids

Refluxed 3g leaf powder with 50ml of methanol for 30 minutes and filtered. Repeated the above process twice. Evaporated the methanol from the filtrate and shaked the residue with 25ml hot water. The above aqueous extract was shaked with 25ml ethyl acetate. Transferred the above solution to a separating funnel and collected the ethyl acetate layer in a previously weighed flask (w_1). Repeated the above process with 20ml, 15ml hot water and ethyl acetate. Continued the same process till the ethyl acetate layer turned colourless. Distilled off the ethyl acetate and evaporated to dryness. Weighed the final residue (w_2). Total flavonoids was estimated in all the seven leaf samples. Percentage of flavonoids = w_2-w_1 x100 /weight of the sample [6].

Table 1. Total polyphenol and flavonoid content of the seven plants under study

Plant name	Total polyphenolic content (%) Mean ± SD	Total flavonoid content (%) Mean ± SD
Asclepias curassavica L.	0.758 ± 0.03	2.73 ± 0.18
Calotropis gigantea (L.) R.Br.	0.13 ± 0.02	1.28 ± 0.06
Gymnema sylvestre (Retz.) R.Br.	0.931 ± 0.12	2.19 ± 0.13
Holostemma ada-kodien Schult.	0.9575 ± 0.08	2.94 ± 0.16
Pergularia daemia (Forsk.) Chiov.	1.524 ± 0.14	4.41 ± 1.02
Tylophora indica (Burm.f.)Merrill.	1.03 ± 0.12	3.21 ± 1.03
Wattakaka volubilis (L.f.) Hasskarl	0.44 ± 0.02	2.42 ± 0.05

Values are mean ± SD of three determinations

Table 2. Antioxidant activity of the seven plants under study

Plant name	IC_{50} value (µg/ml)
Asclepias curassavica L.	170.6
Calotropis gigantea (L.) R.Br.	486.4
Gymnema sylvestre (Retz.) R.Br.	199.526
Holostemma ada-kodien Schult.	158.49
Pergularia daemia (Forsk.) Chiov.	147.7
Tylophora indica (Burm.f.)Merrill.	181.97
Wattakaka volubilis (L.f.) Hasskarl	179.64

IC_{50} value of Ascorbic acid is 8.91µg/ml

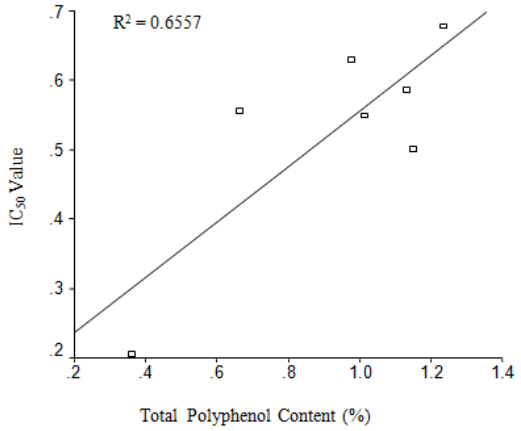

Fig. 1. Correlation coefficient (R^2) of Antioxidant Capacity and Total Polyphenol Content

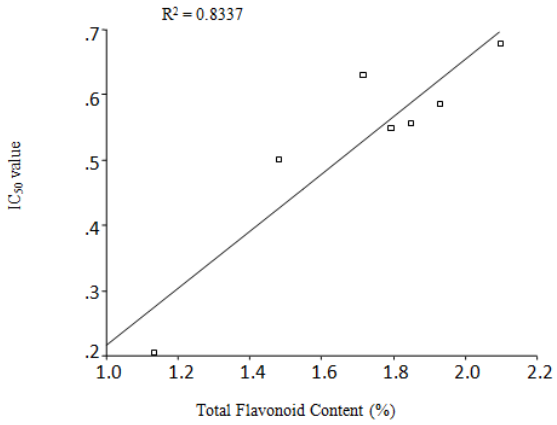

Fig. 2. Correlation coefficient (R^2) of Antioxidant capacity and flavonoid content

Antioxidant capacity determination

Free radical scavenging activity was determined by DPPH (1,1-diphenyl-2-picryl- hydrazyl) assay method [7]. The activity is expressed as IC_{50} (i.e. the concentration of the test solution required to give 50% decrease in absorbance compared to that of a control solution). The decrease in absorbance of DPPH at its absorbance maximum of 516nm is proportional to the concentration of free radical scavenger added to the DPPH reagent solution. IC_{50} was calculated by plotting the linear regression calibration curve of log of concentration of test solution on X-axis against percentage reduction in absorbance on Y-axis. Ascorbic acid pure was used as a standard.

Statistical Analysis

The analysis was carried out using statistical package SPSS

RESULTS AND DISCUSSION

The highest total polyphenolic content and flavonoid content was recorded in *Pergularia daemia* (Forsk.) Chiov. (1.524 ± 0.14 % and 4.41 ± 1.02 % respectively), followed by *Tylophora indica* (Burm.f.)Merrill.(1.03 ± 0.12 % and 3.21 ± 1.03 % respectively) The lowest total polyphenolic content and flavonoid content was found in *Calotropis gigantea* (L.) R.Br. (0.13 ± 0.02 % and 1.28 ± 0.06 % respectively) (Table 1) Table 2 shows the comparative data of DPPH free radical scavenging activity , as determined by the IC_{50} values of the different leaf extracts. IC_{50} value is inversely related to the activity. Highest activity was found in *Pergularia daemia* (Forsk.) Chiov. (147.7 µg/ml ***)*** followed by *Holostemma ada-kodien* Schult. (158.49 µg/ml) and the lowest activity was found in *Calotropis gigantea* (L.) R.Br. (486.4 µg/ml). Among these plants studied the one which had the highest polyphenolic and flavonoids contents, was also found to possess the best antioxidant activity and the one which had the lowest polyphenolic and flavonoids contents, was also found to possess the least antioxidant activity. Figure 1 - shows the relationship between antioxidant activity and total polyphenol content. In order to make a normal distribution the square root of the variables on the X axis and the reciprocal percentage of the variables on the Y axis were taken. The transformed variables give a linear relationship with correlation coefficient (R^2) value 0.6557 which shows that these two variables are correlated. Figure 2 shows the relationship between antioxidant activity and flavonoid content. In order to make a normal distribution the square root of the variables on the X

axis and the reciprocal percentage of the variables on the Y axis were taken. Correlation coefficient (R^2) value 0.8337 shows that these two variables are highly correlated indicating that flavonoids present in these plants may be responsible for the antioxidant property.

CONCLUSION

DPPH assay is quick, reliable and reproducible method widely used to test the ability of compounds as free radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts [8]. In the present study, it was observed that the antioxidant activities more or less had direct correlation with quantities of total polyphenols thus agreeing with the earlier reports [9]. The strong correlation coefficient (R^2) value 0.8337 suggests that flavonoid compounds present in these plants contribute well to the antioxidant capacity. The results obtained in the present study indicates that the leaves of these medicinal plants have great importance as therapeutic agents in preventing oxidative stress related degenerative diseases. However, conformation of its activity in *in vivo* models should be carried out. Such antioxidants could replace synthetic toxic antioxidant.

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