Journal of Applied Pharmaceutical Science Vol. 3 (03), pp. 109-111, March, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.30321
ISSN 2231-3354

Radical scavenging and *In-Vitro* Hemolytic Activity of Aqueous Extracts of Selected Acacia Species

Sulaiman C T[1, 2] and Gopalakrishnan V K[2]
[1]Centre for Medicinal Plants Research, Arya Vaidya Sala Kottakkal, Malappuram, Kerala-676503
[2]Department of Biochemistry, Karpagam University, Coimbatore, Tamil Nadu, India.

ARTICLE INFO

Article history:
Received on: 25/01/2013
Revised on: 19/02/2013
Accepted on: 03/03/2013
Available online: 30/03/2013

Key words:
Acacia, Phenolics, DPPH, Hemolytic activity.

ABSTRACT

The aqueous extracts of three *Acacia* species were screened for their free radical scavenging and *in vitro* hemolytic activity. The total phenolic content was also determined in all the three species. The total phenolics were estimated spectrophotometrically based on the reduction of phosphomolybdate ion of Folin-Ciocalteu reagent and was expressed as milligram equivalent of Gallic acid. The radical scavenging activity was evaluated by DPPH assay. *Acacia nilotica* showed maximum radical scavenging activity with EC_{50} 12.5. Hemolytic activity of the aqueous extracts was screened against normal human erythrocytes. Aqueous extract of *A. leucophloea* possessed minimum hemolytic activity where as *A.nilotica* showed highest hemolytic activity.

INTRODUCTION

Acacia is the second largest genus in the Leguminosae family, comprising more than 1200 species worldwide, with members found in almost all habitats. This species contains variety of bioactive components such as phenolic acids , alkaloids ,terpenes, tannins and flavonoids which are responsible for numerous biological and pharmacological properties like hypoglycemic, anti-inflammatory, anti-bacterial, anti-platelet aggregatory, anti-hypertensive, analgesic, anti-cancer, and antiatherosclerotic due to their strong antioxidant and free radical scavenging activities. Phenolics are largest group of phytochemicals and accounts for most of the antioxidant activity in plants or plant products (Sulaiman and Gopalakrishnan, 2011). *In vitro* hemolytic activities are becoming a new area of research in drug lead discovery. Researchers are exploring ethno botanically important plants to find out potential natural products with antiaggregant action. These studies are important because some patients have become resistant to the already existing drug e.g. aspirin (Undas *et al*, 2007) and/ or conventional medication in association with medicinal plant formulations. This is posing a serious problem to the society. Moreover the constant use of synthetic drugs is leading the society to face great danger. In recent years, many antiplatelet aggregating agents have been isolated from plants and have demonstrated potent activity (Dong *et al*, 1998).

** Corresponding Author*
Phytochemistry Division, Centre for Medicinal Plants Research, Arya Vaidya Sala Kottakkal, Malappuram, Kerala-676503
+914832806210/+919447382799

MATERIALS AND METHODS

Plants (*Acacia nilotica* and *Acacia leucophloea*) were collected from Gundelpet District of Karnataka state, India, *Acacia catechu* was collected from Herb garden of Arya Vaidya Sala, Kottakkal, Kerala, India, and all the materials were authenticated by Taxonomy Division Centre for Medicinal Plants Research (CMPR), Arya Vaidya Sala Kottakkal, Kerala, India.

Preparation of Plant Extract

10 g of dry powder of each, *A. catechu, A. leucophloea and A.nilotica* was taken and suspended in 100 mL of distilled water and subjected extraction by refluxing. The extract obtained was filtered and the process was repeated for four days. The resulting filtrates were pooled for further processing. This pooled aqueous ethanolic extract was concentrated to 50 mL on rotavapour and it is taken for the study.

Total Phenolic Content (TPC)

The assay was based on the reduction of phosphomolybdate ion of Folin-Ciocalteu reagent by the phenolate ion of sample (Singleton *et al*, 1956). A desired amount of plant extract, distilled water and 1 N Folin-Ciocalteu reagent was taken into a tube and mixed thoroughly. After an interval of 3 min, 2 ml of 2% sodium carbonate solution was added and the mixture was allowed to stand for 30 min with intermittent shaking. The absorbance of the mixture was measured at 750 nm using spectrophotometer (Shimadzu, Japan). Different Gallic acid standards (2, 5, 7, 10, and 15 µg/ml) were used for obtaining a standard curve. The total phenolic content was expressed as Gallic acid equivalents (GAE) in milligrams per gram of sample.

DPPH Assay

The method described by Tepe *et al.* 2005 was used with minor modifications. One ml of 500 µM DPPH in methanol was mixed with equal volume of the extract solution in phosphate buffer (pH 7.4). The mixture was slightly shaken and kept in dark for 20 minutes. The absorbance at 517 nm was monitored in presence and absence of different concentrations of the extracts. BHA was used as standard.

Hemolytic assay

Hemolytic assay was carried out by adopting the method of Bulmus *et al*, 2003. Freshly collected human red blood cells were taken and washed three times by 150 mM NaCl (2500 rpm for 10 minutes). The serum was removed and the cells were suspended in 100 mM sodium phosphate buffer. Four different concentrations (50 µg, 100 µg, 150 µg and 200 µg) of extracts were mixed with 200 mL of RBC solutions and the final reaction mixture volume was made up to 1 ml by adding sodium phosphate buffer. The reaction mixture was then placed in water bath for 1 hour at 37°C. After the incubation time the reaction mixture was centrifuged again at 2500 rpm for 15 minutes. The supernatant was collected and the optical density was measured at 541 nm keeping sodium phosphate buffer as blank. Deionised water was used as a positive control. The experiment was done in triplicate and mean ± S.D. was calculated.

Percentage hemolysis =

$$\frac{(\text{Absorbance of sample- Absorbance of blank}) \times 100}{\text{Absorbance of positive control}}$$

RESULTS AND DISCUSSION

The total Phenolic content of three plants is listed in Table 1.1.The highest phenolic content is in *Acacia nilotica* (9.82 mg GAE). The total phenolic content varied from 6.5 to 9.82 GAE mg/g. The antioxidant capacity of extracts from selected plants was determined by DPPH assay and was compared to their total phenolic content. Hydrogen donating property of the polyphenolic compounds is responsible for their inhibition of free radical. In 1'-1' diphenylpicryl-hydrazyl radical scavenging assay, (table.1.2). All the extracts showed significant radical scavenging activity. *Acacia nilotica* showed maximum activity with EC_{50} 12.5. It was observed that the free radical scavenging activity increased with the increase of phenolic compound content. It was already reported that positive correlation between free radical scavenging activity and total phenolic compound. The linear relation between phenolic content and antioxidant activity indicates that the phenolic compounds might be the major contributors towards the free radical scavenging activities of plant extracts. The present investigation showed that the selected three species are rich source of naturally occurring antioxidant phenolic compounds.

Hemolytic activity of the aqueous extracts of different Acacia species were screened against normal human erythrocytes. Extracts exhibited low to mild hemolytic effect toward human erythrocytes. Hemolytic activity of the plant is expressed in % hemolysis and reported as mean ± standard deviation of three replicates. Result indicated that aqueous extract of *A. leucophloea* (at dose 50 µg/ml) possess minimum hemolytic activity (2.6 ±0.11%) where as *A.nilotica* (at dose 200 µg/ml) possess highest hemolytic activity (8.9 ±0.16) Hemolytic percentage was found to be increasing with increase in dose (Table 1.3).

Table. 1.1: Total phenolics (mg GAE).

Acacia catechu	8.68
Acacia leucophloea	6.5
Acacia nilotica	9.82

Table.1.2: DPPH radical scavenging assay.

Sl No	Sample	EC_{50}
1	*Acacia catechu*	13.8
2	*Acacia leucophloea*	17.6
3	*Acacia nilotica*	12.5
4	BHA	6.5

Table. 1.3: *in vitro* Hemolytic activities of Acacia species.

Sl No	Sample	% of Hemolysis			
		50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml
1	*A.catechu*	3.6 ±0.24	4.2 ±0.28	4.8 ±0.88	6.7 ±0.12
2	*A.nilotica*	4.8 ±0.05	6.3 ±0.46	7.8 ±0.62	8.9 ±0.16
3	*A. leucophloea*	2.6 ±0.11	3.5 ±0.26	4.8 ±36	7.3 ±0.24

CONCLUSION

The three *Acacia* species taken for the present studies are used as medicinal plants. The present study evaluated the total phenolic content and its relation with radical scavenging activity of aqueous extracts of selected *Acacia* species. A.nilotica showed maximum radical scavenging activity and hemolytic activity compared to two other species. The antioxidant effect of plant products is mainly due to phenolic compounds, such as flavonoids and phenolic acids. DPPH radical scavenging activity was found to be increasing with increase of total phenolic content. The result of hemolytic activity revealed that the aqueous extracts possess very less hemolytic activity.

ACKNOWLEDGEMENTS

The authors are thankful to the authorities of Arya Vaidya Sala Kottakkal for extending the facilities and TATA Trust, Mumbai for financial assistance.

REFERENCES

Bulmus V., Woodward M., Lin L., Murthy N., Stayton P., Hoffman A. A new pH-responsive and glutathione-reactive, endosomal membrane-disruptive polymeric carrier for intracellular delivery of biomolecular drugs. Journal of Controlled Release. 2003; 93: 105-120.

Dong H., Chen S X., Xu H X., Kadota S., Namba T. A new antiplatelet diarylheptanoid from *Alpinia blepharocalyx*. Journal of Natural Products. 1998; 61:142-144.

Singleton V L., Rossi J r, JA. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965;16:144-158.

Sulaiman C T., Gopalakrishnan V K. Spectrophotometric determination of antioxidant potential of selected acacia species. Journal of Tropical Medicinal Plants. 2011;12: 157-161.

Tepe B., Daferera D., Sokmen A., Sokmen M., Polissiou M.,Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). Food Chemistry. 2005; 90: 333–340.

Undas A., Brummel-Ziedins E., Mann K G. Antithombotic properties to aspirin: beyond strictly antiplatelet actions. Blood. 2007;109: 2285-2292.

How to cite this article:

Sulaiman C T and Gopalakrishnan V K., Radical scavenging and *In-Vitro* Hemolytic Activity of Aqueous Extracts of Selected Acacia Species. J App Pharm Sci. 2013; 3 (03): 109-111.