Pharmacological analysis of hydroethanolic extract of Senna alata (L.) for in vitro free radical scavenging and cytotoxic activities against HepG2 cancer cell line

Article *in* Pakistan Journal of Pharmaceutical Sciences · May 2019

CITATIONS
30

READS
384

6 authors, including:

Mohanasundaram Sugumar
SRM College of Agricultural Sciences SRM Institute of Science and Technology
33 PUBLICATIONS **337** CITATIONS
SEE PROFILE

Victor Doss
PSG College of Arts and Science
32 PUBLICATIONS **198** CITATIONS
SEE PROFILE

Subathra Munuswamy
Aarupadai Veedu Institute of Technology
1 PUBLICATION **30** CITATIONS
SEE PROFILE

Pharmacological analysis of hydroethanolic extract of *Senna alata* (L.) for *in vitro* free radical scavenging and cytotoxic activities against HepG2 cancer cell line

Mohanasundaram Sugumar[1,3]*, Victor Arokia Doss[2], Prasad Maddisetty[1], Magesh Rangaraju[3], Sivakumar Kandhasamy[3] and Subathra Munuswamy[3]

[1]Research & Development Centre, Bharathiar University, Coimbatore, India
[2]Department of Biochemistry, PSG College of arts and Science, Coimbatore, India
[3]Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, Maduranthagam, Kanchipuram, India

Abstract: The main objective of this study was to evaluate the hydroethanolic extract of *Senna alata* for the possible free radical scavenging and cytotoxic properties. Using such hydroethanolic extract, various *in vitro* antioxidant assays at different concentrations were performed and analyzed. In all the assays, plant extract has good inhibitory effect. Ethanolic extract of *Senna alata* was further subjected into cytotoxicity against HepG2 cell line. Accordingly, it was also found that the plant extract has appreciable potency against cancer cell lines.

Keywords: *Senna alata*, antioxidant, lipid peroxidation, antioxidant, anticancer.

INTRODUCTION

The vast section of traditional and folklore medicine is herbal sources. Its core use for both communicable and non-communicable conditions needs to be tested in a scientific manner. For this medicinal plants constitute an option for acute conditions. This will definitely help to develop new drugs. Medicinal plants can meet the need of drug and nutrition which are famous for decades.

A huge range of therapeutics can be generated from the medicinal plants. The folklore therapeutics are inexpensive as they are collected from inexpensive rural sources. The bioactive compounds obtained from the plant kingdom are having significant role in clinical and agricultural research activities. Plant products are the prime and safe choices in folklore treatment practices (Mohanasundaram *et al*., 2011). *Senna alata* (L) of Fabaceae is a shrub which is traditionally used to treat parasitic ringworms, skin diseases and infections. The morphological studies of this plant reveals that it grows around 2-5m in height and possess horizontally spread branches with 10-20 pair of leaflets. Flowers are axillary racemes with short pedicles and green sepals. It has bright yellow coloured petals, short calved with stamens and fruit is thick with quadrangular seeds (Farnsworth and Bunyapraphatsara, 1992). The plant is an alternative form of medication and is free of side effects.

The main aim of this study was to analyze hydroethanolic extract of *Senna alata* leaves for its bioactive compounds with pharmacologically important therapeutic values like antioxidant, free radical scavenging and anticancer properties.

MATERIALS AND METHODS

Collection & authentication of Plant

Senna alata L leaves were collected in and around the areas of Thirukkalukundram Taluk, Kanchipuram District, Tamil Nadu with the help of local community people. The plant sample was further identified and certified by the experts of BSI, India (Plant Identification No.BSI/SRC/5/23/2013-14/Tech.1513).

Preparation of the plant extract

The fresh leaves of *Senna alata* (L) were shade dried and grounded into coarse powder. This coarse powder was kept in air tight container and the extract was prepared using 50% (v/v) hydroethanol. The extract was then condensed to dryness (at 35^{o}C) using rotary evaporator and then used for further studies.

Qualitative analysis of phytochemicals

Qualitative phytochemical analysis was carried by following standard method (Harbone, 1998).

Antioxidant and cytotoxic activity assays

The major assays include DPPH free radical scavenging assay (Blois, 1958), hydrogen Peroxide scavenging assay (Ruch *et al*., 1989), nitric oxide radical inhibition assay (Garratt, 1964), super oxide scavenging activity (Liu *et al*., 1997), hydroxyl radical scavenging activity assay (Elizabeth and Rao 1990) and *in vitro* cytotoxicity by MTT assay (Mossman, 1983) were performed.

**Corresponding author:* e-mail: sbmohan2007@gmail.com

RESULTS

Qualitative analysis of 50% hydroethanolic extract of this plant revealed the presence of major phytoconstituents including glycosides, tannins, alkaloids, flavonoids, quinones, volatile oils, terpenoids, phytosteroids, phenols and other secondary metabolites (Victor *et al*., 2016).

DPPH free radical scavenging assay

This is one of the best assays for determining the antioxidant effect in a time dependent manner (Abeer and Walid, 2008). Extracts of *Senna alata* L leaves showed scavenging activity of DPPH radical (fig. 1), which may be a characteristic feature for its hydrogen bonding ability. Phenolic compounds perform a good antioxidant process (Yen *et al*., 1993).

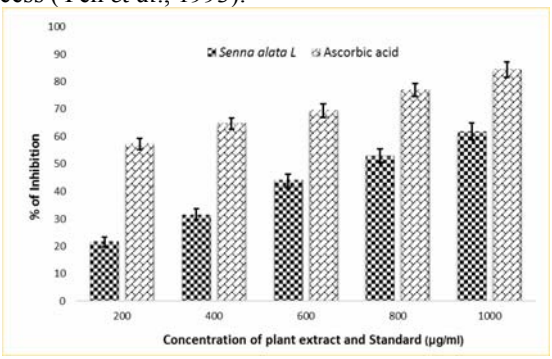

Fig. 1: DPPH Radical scavenging activity of *Senna alata* L extract.

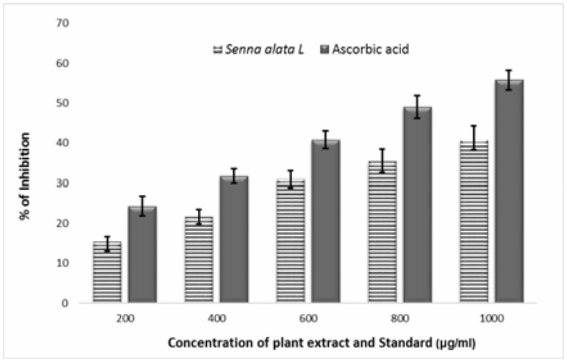

Fig. 2: Hydrogen Peroxide scavenging of *Senna alata* L extract.

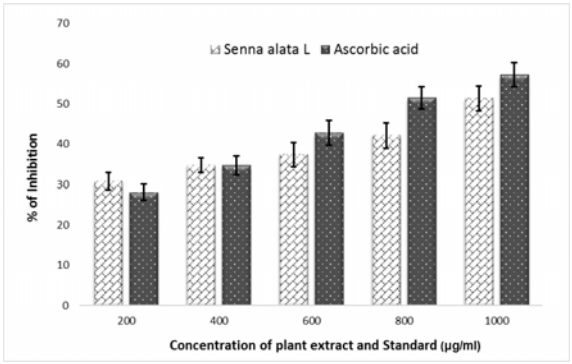

Fig. 3: Nitric oxide radical inhibition assay of *Senna alata* L extract.

The scavenging activity was analyzed by DPPH reducing activity. The % of inhibition by standard Ascorbic acid was 84.38% and *Senna alata* L showed 61.69% of inhibition at 1000µg/ml concentration.

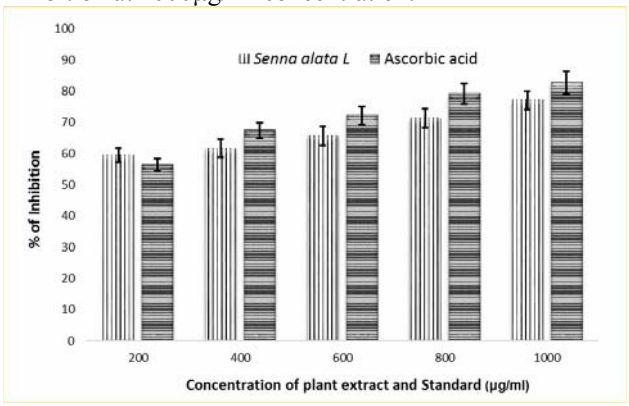

Fig. 4: Super oxide scavenging activity of *Senna alata* L extract.

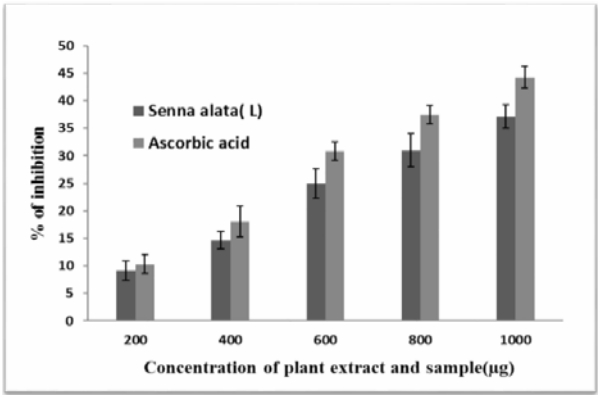

Fig. 5: Hydroxyl radical scavenging activity assay of *Senna alata* L extract.

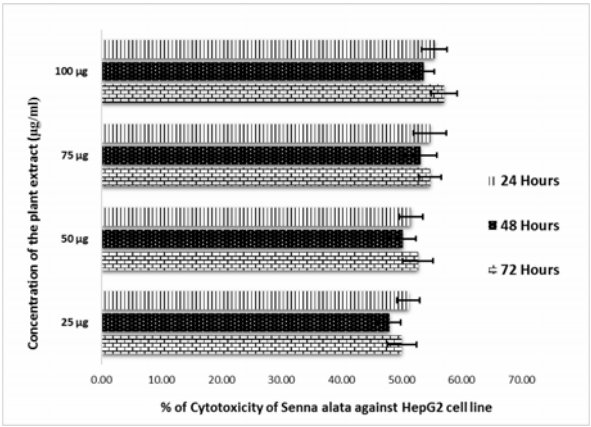

Fig. 6: *In vitro* cytotoxicity assay of *Senna alata* L.

DISCUSSIONS

Hydrogen peroxide scavenging assay

Accordingly, *Senna alata* L showed 40.81% inhibition whereas standard ascorbic acid showed 55.75% inhibition at 1000µg/ml. This dose dependent potency is clearly depicted in the fig. 2.

Nitric oxide radical inhibition assay

The scavenging activity of 50% hydroethanolic extract of *Senna alata* L leaves is shown in fig. 3 whereby it was found effective in dose dependent manner.

S. alata leaf extract is potent scavenger of nitric oxide as observed in this assay. In general, antioxidants inhibit radicals formation by competing with O_2 to react with nitric oxide and also inhibit the generation or leading to least nitric oxide generation (Marcocci *et al*., 1994).

Super oxide scavenging activity

Superoxide radical (O^{2-}) is a reduced form of molecular oxygen and are highly reactive species. They are generated by several biological and photochemical redox reactions. These super oxides radical could be converted into H_2O_2 and other reactive oxygen species in the presence of Super oxide dismutase (Govindarajan *et al*., 2003). Peroxides, hydrogen radicals and nascent oxygen favors oxidative stress in all the major biomolecules (Aruoma, 1998).

Fig. 4 shows superoxide scavenging activity of *Senna alata* L leaves extract. The results indicate that *Senna alata* L has the capability of super oxide radical scavenging activity in a dose dependent manner.

Hydroxyl radical scavenging activity assay

This potency found in the extracts of *Senna alata* L leaves is shown in fig. 5. Hydroxyl radicals induce the peroxidation of unsaturated fatty acids, proteins and DNA (Spencer *et al*., 1996). The plant extracts reduce hydroxyl ions and prevent lipid peroxidation, which might be the cause of antioxidant activities of *Senna alata* L leaf extracts.

Many plants, for example mulberry (fruit extract) are known for their effect to scavenge hydroxyl radicals (Song-Hwan and Hyung-Joo, 2007).

Cytotoxicity assessment by MTT assay

The MTT assay showed significant cell cytotoxicity by the leaf extract after 24hrs, 48hrs and 72hrs along with marked difference in the cell viability. Varied concentration resulted in 57 % of cytotoxicity observed at 100µg/ml concentration after 72hrs of incubation. The other concentrations of the extract ranged between 42-50 % of activity over 72hr duration and significant difference after 24 and 48hr duration period.

From the above results, it is very clear that *Senna alata* L plant is a rich source of bioactive compounds with antioxidant property and cytotoxic activity. Earlier studies (Khare, 2007; Liu *et al*., 2009), had well proved that *Senna alata* contains major pharmacologically important bioactive compounds including phenolics (chrysaphanol, emodin, rhein, aloe-emodin, kaempferol and their glycosides), fatty acids (palmitic, oleic, linoleic acids), terpenoids (β-sitosterol, stigmasterol, campesterol) and anthraquinones (e.g. alatonal, alatinone).

CONCLUSION

The development of phytocompounds in the therapeutic medications by replacing the synthetic compounds. The research carried out herein shows the potentials of *Senna alata* leaves hydroethanolic extract whereby its valiant properties were assayed. Using such hydroethanolic extract, *in vitro* antioxidant studies were performed. In all the assays, plant extract was found to have good potency close to the standard. Ethanolic extract of *Senna alata* was also found to possess good cytotoxicity against HepG2 cell line. Further research on its valuable compounds responsible for the above observed biological activity of *Senna alata* (L) and its potential can be studied by *in vivo* models in future.

ACKNOWLEDGEMENTS

The authors are grateful to Research and Development Centre, Bharathiar University, Coimbatore for approving this project. We are very much thankful to Mrs. Meenakshi Annamalai Director, Karpaga Vinayaga College of Engineering and Technology for the continuous support throughout the completion of this project successfully.

REFERENCES

Abeer T and Walid HE (2008). Characterization of antioxidant activity of extract from *Artemisia vulgaris. Pak. J. Parm. Sci.,* **21**(4): 321-326.

Aruoma OI (1998). Free radical oxidative stress and antioxidants in human health and disease. *J. Am. Oil. Chem. Soc.,* **75**: 199.

Blois MS (1958). Antioxidant determinations by the use of a stable free radical. *Nature,* **29**: 1199-1200.

Elizabeth K and Rao MNA (1990). Oxygen radical scavenging activity of curcumin. *International Journal of Pharmaceutics*, **58**: 237-240.

Farnsworth NR and Bunyapraphatsara N (1992). Thai medicinal plants. Recommended for primary health care system. Prachachon, Bangkok.

Garratt DC (1964). The quantitative analysis of drugs (Vol. 3). Japan: Chapman and Hall Ltd., pp.456-458.

Govindarajan R, Rastogi S and Vijaykumar M (2003). Studies on antioxidant activities of *Desmodium gangeticum. Pharm. Bull,* **26**(10): 1424-1427.

Harbone JB (1998). Phytochemical Methods. A guide to modern techniques of plant analysis 3rd edn. Chapman and Hall, New York, USA, pp.1-150.

Khare CP (2007). Indian Medicinal Plants: An Illustrated Dictionary. Springer-Verlag, Berlin Heidelberg, London, UK, pp.126-127.

Liu A, Xu L, Zou Z and Yang S (2009). Studies on chemical constituents from leaves of *Cassia alata. Zhongguo. Zhong. Yao. Za. Zhi.,* **34**: 861-863.

Liu F, Ooi VEC and Chang ST (1997). Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sciences*, **60**: 763-771.

Marcocci L, Packer L, Droy-Lefai MT, Sekaki A and Cardes-Albert M (1994). Antioxidant action of Ginkgo biloba extracts EGb 761. *Methods Enzymol.,* **234**: 462-475.

Mohanasundaram S, Sivakumar, Karthikeyan, Bhuvaneshwari, Aishwarya, Thirumalai, Pennarasi (2011). Studies on *in vitro* antibacterial, antifungal property and antioxidant potency of *Murraya paniculata*. *Pak. J. of Nutrition,* **10**(10): 925-929.

Mossman, T (1983). Rapid colorimetric assay for cellular growth and survival application to proliferation and cytotoxicity assays. *J. Immunol. Methods,* **65**: 55-63.

Ruch RJ, Cheng SJ and Klaunig JF (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis,* **10**: 1003-1008.

Song-Hwan B and Hyung-Joo S (2007). Antioxidant activity of five different mulberry cultivars in Korea. LWT, **40**: 955-962.

Spencer BF, Dyke SJ, Sain MK and Carlson JD (1996). Nonlinear identification of semi-active control devices. 11th ASCE Engrg. Mech. Spec. Conf. Ft. Lauderdale, Florida.

Victor AD, Mohanasundaram S and Prasad Maddisetty PN (2016). Analysis of hydroethanolic extract of *Senna alata* (L.) to screen bioactive compounds with inhibitory activity on lipid per oxidation, *in vitro* antibacterial and antidiabetic efficacy. *Int. J. Pharma. Sci.,* **6**(1): 1360-1366.

Yen GC, Duh PD and Tsai C (1993). The relationship between antioxidant activity and maturity of peanut hulls. *J. Agric. Food Chem.,* **41**: 256-260