Research Article

Total Phenolic Content and Free Radical Scavenging Activity of *Salacia oblonga* and *Gymnema Sylvestre*: An *In vitro* Comparative Study

Shivani Uday[1], Bheemesh Vangalapati[2], Poornima A Manjrekar[2]*, Anupama Hegde[2]
[1]Department of Biochemistry, St Aloysius college, Mangalore University, Mangalore, Karnataka, India.
[2]Department of Biochemistry, Kasturba Medical College, Manipal University, Mangalore, Karnataka, India.
***Corresponding author's E-mail:** poornima.manjrekar@manipal.edu

Accepted on: 02-07-2015; **Finalized on:** 31-07-2015.

ABSTRACT

The over production of free radicals is involved in triggering many diseases such as diabetes, cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis. Antioxidants derived from plant sources offer resistance against the oxidative stress by way of their free radicals scavenging & lipid peroxidation inhibitory actions. Present study was designed to compare the free radical scavenging activity in relation to the total phenolic content of *G. sylvestre* & *S. oblonga*. Total phenolic content of the extracts was determined by using Folin-Ciocalteu method following a slightly modified method of Singleton by comparing with standard Gallic acid. The ability to scavenge 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical is measured by a decrease in the absorbance at 517 nm as described by Hou comparing with standard Ascorbic acid. Phenolic contents ranging from 35 to 175 and 30 to 85mg of Gallic acid equivalents (GAE) per gram of extract were present in *S.oblonga* & *G.sylvestre* respectively. At 100µg concentration in *S.oblonga* & *G.sylvestre* produced 81.7 & 66.8% of DPPH scavenging activity respectively and the standard ascorbic acid (10µg) produced 91.5% scavenging activity. Based on the study results, both the plant extracts of *S.oblonga* and *G.sylvestre* have significant amounts of phenolic compounds and exhibit free radical scavenging potential. However, between the two, *S.oblonga* plant had better antioxidant properties. Hence these herbal constituents are of adjuvant importance in the management of various free-radical mediated pathological conditions.

Keywords: Oxidative stress, Diabetes, Phenols, Free radical (DPPH), *S.oblonga*, *G.sylvestre*

INTRODUCTION

The over production of free radicals (oxidative stress) is involved in triggering many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as degenerative processes associated with ageing by causing lipid peroxidation[1]. Antioxidants are agents which offer resistance against the oxidative stress by scavenging the free radicals and inhibiting lipid peroxidation or by protecting the antioxidant defence mechanisms. Antioxidant effectiveness is measured in terms of the inhibition of suitable substrate oxidation. Phenolic compounds are known for their scavenging potential due to the presence of hydroxyl groups. It has been discovered that several phenolic antioxidants such as flavonoids, tannins, couramins, xanthones and procyanidins scavenge radicals, dose dependently and therefore are considered as therapeutic agents for free radical pathologies. Plant materials have also been believed to be less toxic with minimum side effects than synthetic drugs. Medicinal plants are easy to access and economic for the marginalised people[2].

Salacia oblonga commonly known as Indian Saptrangi, in Sanskrit –Vairi, belongs to the family *Celastraceae* widely distributed in India, China and Malaysia[3].

It has several traditional uses as anti-microbial[4], antioxidant[5], anti-inflammatory[6], anti-diabetic[7], nephroprotective[8] and anti-mutagenic[9]. Previous studies indicated that diterpenes, sesquiterpenes, triterpenes,triterpenes, glycosides, catechins and polyphenols are present in the *S. oblonga* plant[3].

Gymnema sylvestre is also found throughout India, commonly known as gudmar. A large woody climber belonging to the family Asclepiadaceae. The leaves of the plant are antidiabetic, astringent bitter, thermogenic, antinflammatory anodyne[10]. It contains lupeol, β -amyrin, stigmasterol, saponins, gymnemic acid, resin, tartaric acid, a mixture of triterpene saponins[11]. The dried leaves are used as antiviral, diuretic, antidiabetic, anti-inflamatory, antiallergic, hypoglycemic, hypolipidemic and also for the treatment of obesity, dental caries & rheumatism[11]. Hence, the present *in vitro* study is designed to determine the free radical scavenging activity in relation to the phenolic content in these two plants.

MATERIALS AND METHODS

Plants: Sample of Hydro-alcoholic extract of *S.oblonga* was obtained from Natural Herbal Company, Bengaluru and taxonomically identified by the Botanist.

Preparation of *G.sylvestre* hydro-alcoholic extract

G.sylvestre tender leaves were washed separately and shade dried at room temperature. After drying, the leaves were powdered by an electrical mixer. 50g of dried powdered leaf was suspended in 500 ml of 50% methanol in water and refluxed at 50°C in a soxhlet apparatus for 72 hours. Crude extract was kept for flash evaporation in rotary vacuum flash evaporator at 5 rpm (75°C) for seven hours. Remaining residue was collected from round

bottom flask and dried in heating mantle for one hour to obtain semi-solid form of extract. Total yield was 4.8 grams. The concentrated crude extract was stored at 4°C for further usage.

Chemicals

Gallic acid, 1, 1- diphenyl-2- picrylhydrazyl (DPPH), Folin-Ciocalteu reagent was purchased from Sigma Chemical Co. Ltd. All other chemicals and reagents used were of analytical grade.

Total phenolic content estimation

The total phenolic content of the both plant extracts were determined by using Folin-Ciocalteu reagent following the method described by Singleton[12]. Gallic acid was used as reference standard.

Principle

Phenolic compounds undergo redox reaction with phosphomolydbic acid and phosphotungstic acid present in FolinCiocalteau reagent in alkaline medium and produce blue colored complex.

Procedure

0.5 mL of the plant extract (100 µg/mL) was mixed with 2 mL of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and neutralized with 4 mL of sodium carbonate solution (7.5%, w/v).was mixed with 2 ml of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and neutralized with 4 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature (30±2°C) for 30 min with recurrent shaking for the blue color development. The absorbance was measured at 765 nm using double beam UV-VIS spectrophotometer.

DPPH free radical scavenging

The DPPH free radical activity of both plant extracts was determined by the method of Hou[13]

Principle

Antioxidants react with the stable DPPH radical and convert into2, 2- diphenylpicryl hydrazine. Ability to scavenge the DPPH radical is measured by a decrease in the absorbance[2].

Aliquots containing various concentrations (2–100µg/ml) of *S. oblonga* and *G. sylvestre*: in the final volume of 1 ml were mixed with 1 ml of 0.05 mM DPPH, equal amount of methanol served as control. Ascorbic acid (2- 100µg/ml) was used as standard.

Reaction mixtures were incubated at 37°C for 20 min & the absorbance was recorded at 517 nm. The capability of DPPH free radical scavenging activity was calculated using the following equation

DPPH scavenging effect (%) = [(A0-A1)/A0) × 100], where A0 is the absorbance of the control and A1 is the absorbance of standard/test.

Statistical Analysis

The data was expressed as Mean ± Standard Deviation (S.D.) and between the groups analysis was performed by using ANOVA in SPSS, $p < 0.05$ was considered as significant.

RESULTS

Total phenol Estimation

From the Gallic acid standard graph, the amount of phenols were calculated and expressed as milligrams of phenol per gram of the sample. The total phenolic content was calculated as gallic acid equivalent (GAE) by using the following formula: T=C x V/M. T is the total phenolic content in mg/g of the extracts as GAE, C is the gallic acid concentration obtained from the calibration curve in mg/ml, V is the volume of the extract in ml and M is the weight of the extract in grams. Phenolic content ranging from 35 to 175 and 30 to 85 mg of GAE per gram of extract was present in *S.oblonga* leaf and *G.sylvestre* leaf extract respectively (Figure-1).

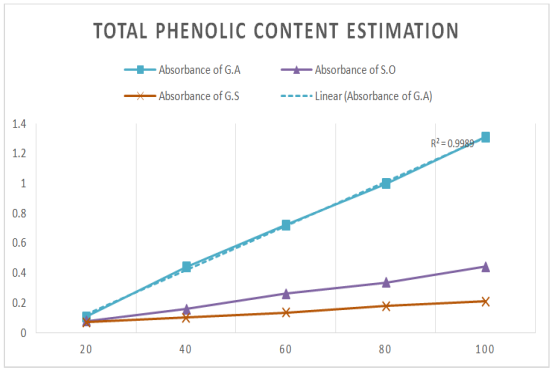

Figure 1: Total Phenolic content estimation

DPPH free radical scavenging activity

Various concentrations (2,4,6,8,10,20,40,60,80,100mg) leaf extracts of *S.oblonga* and *G.sylvestre* were tested for DPPH free radical scavenging activity. It was observed that the test compound scavenged free radicals in a concentration dependent manner. At 100µg concentration *S.oblonga* and *G.sylvestre* produced 81.7 & 66.8% of DPPH scavenging activity respectively. Ascorbic acid (100µg) as standard produced 91.5 % DPPH free radical scavenging activity. (Figure-2).

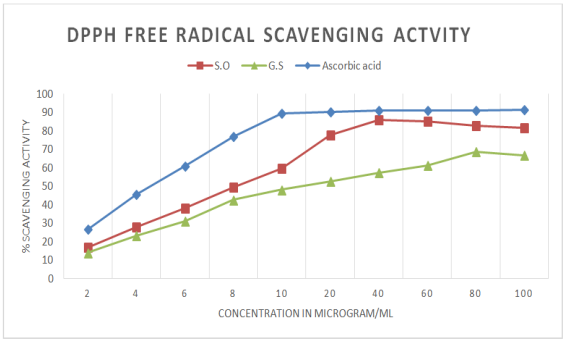

Figure 2: Graphical representation of DPPH free radical scavenging activity

DISCUSSION

In a normal cell there is an appropriate pro-oxidant and antioxidant balance. However, this balance can be shifted towards the pro-oxidant status when production of reactive oxygen species is increased or when levels of antioxidants are diminished. This state is known as oxidative stress.

Free radicals are the chemical bodies that can exist separately with one or more unpaired electrons.

The production of free radicals can bring about many reactions which can lead to extensive tissue damage if the stress is prolonged.

Proteins, lipids and DNA are prone to the free radicals induced damage. Antioxidants act by neutralizing the free radicals or protecting the antioxidant defense mechanisms.

Antioxidant effectiveness is measured in terms of the inhibition of suitable substrate oxidation. Various methods are available for determining free radical scavenging effects.

In this study, hydro-alcoholic extracts of *G.sylvestre* and *S.oblonga leaves* were tested to determine the total phenolic contents and their ability to scavenge the DPPH free radicals by *in vitro* method.

As reported in the earlier studies our results also suggests that *G.sylvestre* leaf has low phenolic content when compared to *S.oblonga* extract.

Due to high phenolic content in *S.oblonga*, it possess potent antioxidant activity as evident from its increased scavenging activity.

There was significant difference ($P < 0.05$) between three groups in their free radical scavenging activity.

Among two extracts, *S.oblonga* exhibited the highest scavenging activity of 81.7% at a concentration of 100 µg/ml.

Further increase in concentrations of *S.oblonga* and Ascorbic acid attained saturation in their scavenging activity whereas *G.sylvestre* shown a concentration dependent activity (Figure-2).

Demonstration of the strong antioxidant activity of this extract would certainly increase its potential as an antioxidant drug which could be used as an adjuvant in the treatment of oxidative stress like type2 diabetes mellitus, ageing cardiovascular diseases etc.

Major phenolic constituents of *S.oblonga* are catechin and polyphenols were also responsible for postprandial glycemic activity which improves cardiac complications in Obese Zucker Rats.

G.sylvestre contain Saponins, anthraquinone and some phenolic compounds, these substances could significantly reduce the level of serum glucose and also produce some antioxidant activity.

CONCLUSION

Based on the results of this *in vitro* study, it can be concluded that both the plant extracts of *S.oblonga* and *G.sylvestre* have significant amounts of phenolic compounds and exhibit free radical scavenging potential. However, between the two, *S.oblonga* plant had better antioxidant properties. Hence these herbal constituents are of adjuvant importance in the management of various free-radical mediated pathological conditions.

REFERENCES

1. Mahmoud A, Saleh, Shavon Clark; Brooke Woodard; SuziatAyomideDeolu-Sobogun, Antioxidants and free radical scavenging activities of essential oils, Ethnicity&Disease, 20, 2010, 78-82.
2. Vangalapati B, Manjrekar P A, Hedge A, Total phenolic content and free radical scavenging activity of *Pterocarpusmarsupium* heartwood and *Tribulusterrestris* dry fruits, Journal of pharmacy research, 8(5), 2014, 610-13.
3. Chawla A, Singh S and Sharma A, *Salaciaoblonga*wall: A review on its pharmacological aspects, International Journal of Research in Pharmaceutical and Biomedical Sciences, 4(4), 2013, 1215-28.
4. Rao MJP, Giri A, Antimicrobial activity of the extract of Salaciaoblonga Wall, Recent Research in Science and Technology, 2(10), 2010, 01-04.
5. Krishnakumar K, Augusti KT, Vijayammal PL, Hypoglycaemic and anti-oxidant activity of Salaciaoblonga Wall. extract in streptozotocin-induced diabetic rats, Indian J PhysiolPharmacol, 43(4), 1999, 510-514.
6. Ismail TS, Gopalakrishnan S, Begum VH, Elango V, Anti-inflammatory activity of Salaciaoblonga Wall. and Azimatetracantha Lam, J Ethnopharmacol, 56(2), 1997, 145-152.
7. Nakata K, Taniguchi Y, Yoshioka N, Yoshida A, Inagawa H, Nakamoto T, Yoshimura H, Miyake S, Kohchi C, Kuroki M, Soma G, A mixture of Salaciaoblonga extract and IP-PA1 reduces fasting plasma glucose (FPG) and low-density lipoprotein (LDL) cholesterol levels, Nutr. Res. Pract, 5(5), 2011, 435–42.
8. Palani SS, Raja SS, Kumar S, Nirmal SN, Kumar B, Senthil BS,Nephroprotective and antioxidant activities of Salaciaoblonga on acetaminophen-induced toxicity in rats, Natural product research, 2011,25(19), 1876-1880.
9. Navneet KS, Biswas A, Rabbani SI, Devi K, Khanam S, Hyrdoalcoholic Root Bark Extract of Salaciaoblonga Prevented Mitomycin-C Induced Sperm Abnormality in Wistar Rats, Pharmacognosy magazine, 5(19), 2009, 254-259.
10. Gupta S, Pramanik O, Tiwari N, Thacker, Immunomodulatory Activity of *Gymnemasylvestre* leaves, Internet Journal of Pharmacology, 8, 2009, 2.
11. Rachh PR, Patel SR, Hirpara HV, Ruparelia MT, Rachh BR, Bhargava BS, Patel BM, Modi DC, *In vitro* evaluation of antioxidant activity of *Gymnemasylvestre*, J.Bio- PlantBiol, 54(2), 2009, 141–8.

12. Singleton VL, Orthofer R, Lamuela-Raventos RM, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Methods Enzymol, 299, 1999, 152-78.

13. Hou W C, Lin R D, Cheng K T, Hung Y T, Cho C H, Chen C H, Hwang S Y, Lee M H, Free radical scavenging activity of Taiwanese native plants, Phytomedicine, 10, 2003, 170-75.

Source of Support: Nil, **Conflict of Interest:** None.