ejpmr, 2016,3(9), 416-421.

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF ANTIOXIDANT ACTIVITY OF RESINS OF BOSWELLIA SERRATA ROXB. EX COLEBR., COMMIPHORA MUKUL (HOOKS EX STOCKS) ENGL., GARDENIA RESINIFERA ROTH. AND SHOREA ROBUSTA GAERTN.

Ujwala C. Bapat[1]*, Smita H. Gaurea[1] and Alka Bapat[2]

[1]Department of Botany, St. Xavier's College, Mahapalika Marg, Mumbai 400 001, Maharashtra State, India.
[2]Seth GSMC, Parel, Mumbai 400012, Maharashtra State, India.

Corresponding Author: Ujwala C. Bapat

[1]Department of Botany, St. Xavier's College, Mahapalika Marg, Mumbai 400 001, Maharashtra State, India.

Article Received on 10/07/2016 **Article Revised on 30/07/2016** **Article Accepted on 20/08/2016**

ABSTRACT

Resins are amorphous mixtures of essential oils, oxygenated products of terpenes and carboxylic acids and are secreted as exudates from specialized structures in a wide range of plants. They probably function as plant defenses. Resins are insoluble in water but usually dissolve in alcohol, ether or carbon di sulphide and other solvents. The well known resins of *Boswellia serrata* Roxb. ex Colebr., *Commiphora mukul* (Hooks ex Stocks) Engl., *Gardenia resinifera* Roth. and *Shorea robusta* Gaertn. were selected to evaluate their antioxidant activity. The resins of these plants have been used traditionally for treatment of various diseases like rheumatism, obesity, hyperlipidemia and skin ailments. The antioxidant activity assays performed were - free radical scavenging activity using DDPH proposed by Mothlanka et al, total antioxidant capacity using phosphomolybdenum reagent described by Prito et al and reducing power assay adopted by Oyaizu. As phenolics and flavonoids are known antioxidants, the antioxidant activity of these resins was correlated with their phenolic and flavonoid contents. Total phenolic and flavonoid contents were determined by the standard methods. All the resin samples exhibited presence of phenolics and flavonoids and antioxidant activity. The highest antioxidant activity was found in *G. resinifera* and C. mukul. *Shorea robusta* showed highest flavonoid content. There was a positive correlation between the total antioxidant capacity and total phenolic and flavonoid content in all the resin extracts. The IC_{50} values obtained indicated that the resin of *G. resinifera* has the maximum antioxidant potential.

KEYWORDS: Resins, *Boswellia serrata*, *Commiphora mukul*, *Gardenia resinifera*, *Shorea robusta*, antioxidant activity, phenols, flavonoids.

INTRODUCTION

Antioxidants are chemical substances that can donate an electron to the free radical and convert it to a harmless molecule. They may reduce the energy of the free radical suppress radical formation or break chain propagation or repair damage and reconstitute membrane.[1] Production of certain free radicals in the human body such as superoxides, hydrogen peroxide, hydroxyl and nitric oxide radicals are the consequence of body's natural metabolic processes. This can trigger the onset of many diseases such as cancer, liver diseases, rheumatoid arthritis.[2] Natural antioxidants such as vitamin C, E, carotenoids, phenolic compounds, etc. that are present in plants are responsible for inhibiting or preventing the deleterious stress exerted by the reactive oxygen species (ROS).[3] Resins are formed as oxidation products of various essential oils and are very complex and varied in chemical composition. The resin is usually secreted in definite cavities or passages. It frequently oozes out through the bark and hardens on exposure to air. Resinous substances may occur alone or in combination with essential oils or gums.[4] Resins, unlike gums, are insoluble in water, but they dissolve in ether, alcohol and other solvents.

Resin production is widespread in nature. They are reported from plants of Pinaceae, Araucariaceae, Umbelliferae, Dipterocarpaceae, Burseraceae, Rubiaceae and Sterculiaceae and are traditionally used to cure various ailments. The resin of *Shorea robusta* belonging to family Dipterocarpaceae is traditionally used in treatment of lumbago and rheumatic pain.[5,6] Tariq et.al reported the analgesic activity of the ethanolic extract of resin of *S. robusta* in experimental animals.[7] The resin of *Boswellia serrata* is reported to be stimulant, antiseptic and useful in skin diseases and rheumatism.[8] Gupta et.al (2011) have done clinical evaluation of *B. serrata* resin in the management of osteoarthritis. Resin of *Commiphora mukul* is used in Ayurvedic medicine in the treatment of rheumatism, obesity and atherosclerosis.

A double blind clinical trial of fraction A of *C. mukul* resin conducted on 84 obese subjects revealed that it was a potent hypolipidaemic agent which reduced serum cholesterol, total lipids and triglycerides significantly.[9] Ramesh et.al. (2011) studied antihyperglycemic and antioxidant activities of alcoholic extract of resin from the same plant.[10] The resin of *Gardenia resinifera* is used as a repellent to keep off flies.[11] and is also an expectorant, carminative, antispasmodic and stimulant.[12] Antioxidants are the substances which get oxidized and prevent the oxidation of components of the cell. They also protect the cells by scavenging the free radicals formed in the cell.

Since resins have been used traditionally and in the ayurvedic preparations for treating various diseases, studies were carried out to evaluate the antimicrobial activity and the antioxidant capacity of the said resins. This paper deals with the antioxidant capacity of the resins of *B. serrata, C. mukul, G. resinifera, S. robusta*.

MATERIALS AND METHODS

Collection of plant resins and their morphological study

The resins of *Boswellia, Commiphora, Gardenia* and *Shorea* were collected from wild. Herbarium sheets of the twigs along with the respective resins were prepared. Morphology of plant specimens as well as the resins was studied and correct identity of the specimens was established by referring floras and herbarium specimens in Blatter Herbarium, St. Xavier's College, Mumbai.

Preparation of resin extracts

1 % extract was prepared by soaking overnight, 0.1 g of resin in 10 ml of Ethanol. The extract was then filtered through Whatman No. 1 filter paper and then evaporated to dryness. The residue collected was dissolved in Ethanol to obtain the required concentrations. The extracts were prepared in triplicates.

Assay for antioxidant activity

Determination of total antioxidant capacity

The method described by Prieto *et al* (1999) was used to determine the total antioxidant capacity of the extracts.[13] The antioxidant capacity was expressed as ascorbic acid equivalent (AAE). The total antioxidant capacity was determined by phosphomolybdenum method, which is based on the reduction of Mo^{+6} to Mo^{+5} by the sample analyte and the subsequent formation of green phosphate-Mo^{+5} complex with a maximum absorption at 695 nm was estimated.[14]

Reducing power assay

The method proposed by Oyaizu et al (1986) was used for the reducing power assay.[13] Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity.[15]

Antioxidants which have reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form Fe^{2+}-Ferrozine complex that has an absorption maximum at 700 nm.[16] The reducing power of the extracts was expressed in terms of absorbance and compared with that of ascorbic acid standard.

DPPH radical scavenging activity

DPPH (2, 2-diphenyl-1-picryl hydrazyl) is the most commonly used stable free radical, to test the potential of compounds as free radical scavengers of hydrogen donors and to investigate the antioxidant activity of plant extracts.[17] Antioxidant molecules when incubated, react with DPPH and convert it into 2, 2 -diphenyl-1-picryl hydrazine, which is a measure of the scavenging potential of plant extracts and can be measured at 520 nm. The DPPH radical scavenging activity was carried out as per the method used by Motlhanka *et al* (2008)[18] and was calculated using the following equation:

DPPH scavenging activity (%) = $[(A_o-A_1)/A_o] \times 100$

where, A_o was the absorbance of the blank (i.e. only DPPH solution, no sample) and A_1 was the absorbance in presence of the test compound / ascorbic acid standard.

Estimation of total phenolic and flavonoids

The total phenolic and flavonoid contents of the extracts were estimated by modified Folin-Ciocalteau method which was proposed by Eberhardt et al (2000)[19] while, the total flavonoid content was estimated by Aluminium chloride method which was described by Zhishen et al (1999).[19] The total phenolic content was expressed as mg of gallic acid equivalent per mg of residue and the flavonoid content as mg of rutin equivalent per mg of residue.

STATISTICAL ANALYSIS

Linear regression analysis was used to calculate IC_{50} values for DPPH radical scavenging assay. The correlation analysis was carried out to determine the relationship between the antioxidant capacity and total phenolic and flavonoid contents.

RESULTS AND DISCUSSION

Antioxidant activity assays

Determination of total antioxidant capacity

The standard graph of ascorbic acid for total antioxidant capacity is given below (Figure 1)

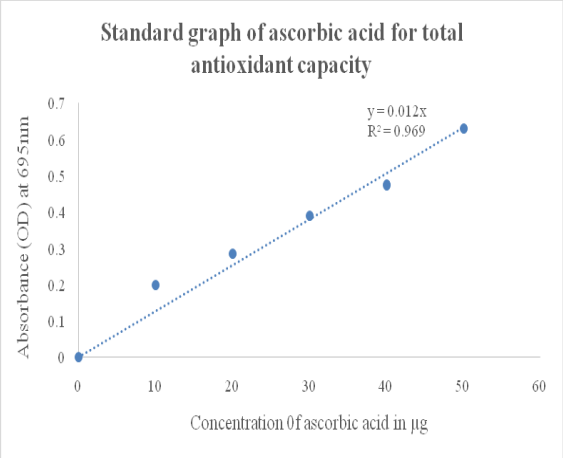

Figure 1: Standard graph of ascorbic acid for total antioxidant capacity

It can be seen (referring to R^2 value) that there is a strong +ve correlation between the concentration of ascorbic acid and the absorbance. The total antioxidant capacity of the extracts is shown in Figure 2

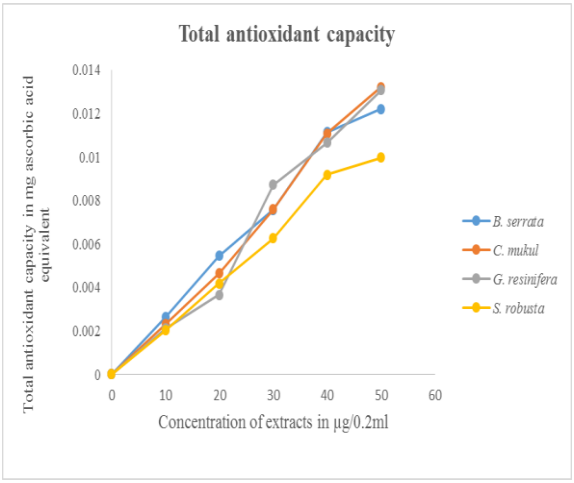

Figure 2: Total antioxidant capacity of extracts

Figure 2 indicates that as the concentration of extracts increases, there is an increase in the total antioxidant capacity. The lowest antioxidant capacity was recorded for *S. robusta* (0.010±0.461 mg AAE/ 50µg of residue). The highest antioxidant capacity was observed for the resin extracts of *C. mukul and G. resinifera* (0.013±0.0516 mg AAE/ 50µg of residue).

This antioxidant activity might be attributed to the phytochemicals such as phenolics and flavonoids.

Reducing power assay

Reducing power may serve as a significant reflection of the antioxidant activity. The results of reducing power assay are as follows.

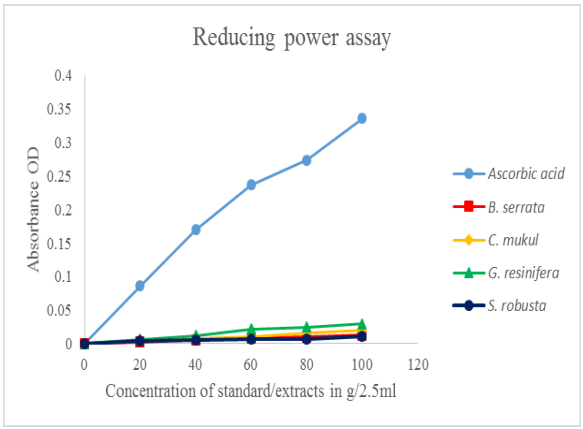

Figure 3: Reducing power assay

Figure 3 shows that as the concentration of standard / resin extracts increases, there is an increase in the absorbance indicating the increase in the reducing power. Among the extracts the highest reducing power was observed with the resin extract of *G. resinifera* (8.63 % of ascorbic acid standard) and the lowest reducing power was observed for the resin extract of *S. robusta* (3.27 % of ascorbic acid standard). The reducing power of extracts of *C. mukul* and *B. serrata* were 5.95 and 3.87 % of ascorbic acid standard respectively.

DPPH radical scavenging activity

The assay of DPPH radical scavenging activity of ascorbic acid (standard) and the extracts shows following results.

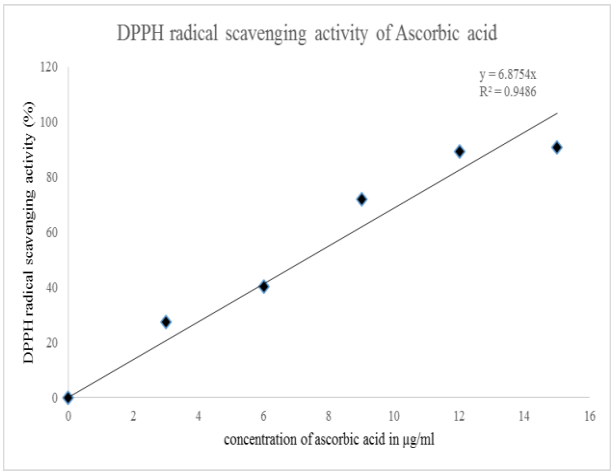

Figure 4: DPPH radical scavenging activity of ascorbic acid

A strong +ve correlation between the concentration of ascorbic acid and the DPPH radical scavenging activity is observed in the above graph (R^2 = 0.9486).

The DPPH radical scavenging activity of resin extracts and the IC_{50} values are given in the following table.

Table 1: DPPH radical scavenging activity of extracts of *B. serrata, C. mukul, G. resinifera, S. robusta* and the IC_{50} values.

Ascorbic acid/ Extract	Concentration in µg/ml	DPPH radical scavenging activity(%)	IC_{50} µg/ml
Ascorbic acid	3	27.36±1.79	7.27
	6	40.20±1.63	
	9	71.90±3.92	
	12	89.30±0.25	
	15	90.76±0.39	
B. serrata	200	2.80±0.08	5882.35
	400	4.00±0.15	
	600	5.28±0.38	
	800	6.33±0.50	
	1000	8.28±0.14	
C. mukul	200	12.24±3.51	843.17
	400	35.87±3.19	
	600	40.77±3.02	
	800	47.97±0.36	
	1000	50.80±0.87	
G. resinifera	200	24.68±0.80	458.30
	400	51.88±0.75	
	600	76.13±1.16	
	800	91.06±0.72	
	1000	95.89±0.09	
S. robusta	200	3.28±1.36	4000
	400	6.52±0.71	
	600	8.33±0.42	
	800	9.38±0.84	
	1000	11.66±0.54	

From the above table, it is clear that as the concentration of extracts increases, there is an increase in the DPPH radical scavenging activity. The highest IC_{50} value was observed for the extract of *B. serrata* (5882.35 µg/ ml) indicating the lowest DPPH radical scavenging activity. The lowest IC_{50} value was observed for the extract of *G. resinifera* (458.30 µg/ ml) indicating the highest DPPH radical scavenging activity. IC_{50} values for *S. robusta* and *C. mukul* were (4000 and 843.17 µg/ ml) respectively. The IC_{50} values of all the extracts were greater as compared to that of ascorbic acid (7.27 µg / ml), indicating very low DPPH radical scavenging activity of the extracts.

Estimation of total phenols and flavonoids

The total phenolic and flavonoid contents of the extracts are shown in Figure 5 and 6.

The highest phenolic content was observed in the *G. resinifera* (0.092 mg of gallic acid equivalents per mg of residue). The lowest phenolic content per mg of residue was observed in the resin extract of *B. serrata* (0.021 mg of gallic acid equivalents / mg residue). The total phenolic content of *C. mukul* was 0.029 and of *S. robusta* was 0.028 mg of gallic acid equivalents / mg of residue (Figure 5).

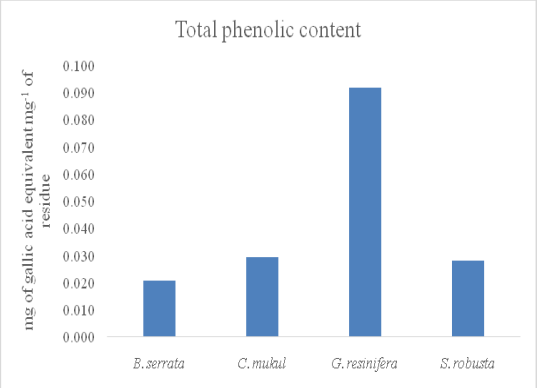

Figure 5: Total phenolic content of the extracts (expressed as mg of gallic acid equivalent mg^{-1} of residue)

The highest flavonoid content was observed in the resin extract of *S. robusta* (0.588 mg of rutin equivalents per mg of residue) and the lowest flavonoid content was found in the extract of *G. resinifera* (0.239 mg of rutin equivalents per mg of residue). The total flavonoid content of *C. mukul* was 0.302 and of *B. serrata* was 0.286 mg of gallic acid equivalents per mg of residue.

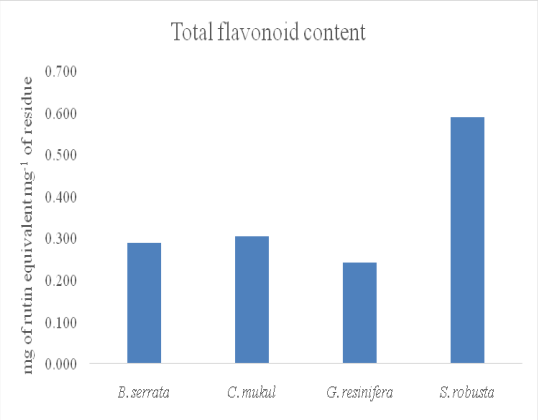

Figure 6: Total flavonoid content of the extracts (mg of rutin equivalent mg^{-1} of residue)

The total antioxidant capacity was correlated with the total phenolic and flavonoid contents. The results are given in Table 2

Table 2: Correlation between the total antioxidant capacity and the total phenolic and flavonoid contents

Plant	R^2 value (phenolic content)	R^2 value (flavonoid content)
Boswellia serrata	0.9565	0.9269
Commiphora mukul	0.8984	0.9823
Gardenia resinifera	0.9691	0.9973
Shorea robusta	0.9315	0.9705

There is a +ve correlation between the total antioxidant capacity and the phenolic content of the resin extracts of *C. mukul* and *S. robusta* and between the antioxidant capacity and the flavonoid content of the resin extract of *B. serrata*.

There is a strong +ve correlation between the total antioxidant capacity and the phenolic content of *B. serrata* and *G. resinifera* and between the antioxidant capacity and the flavonoid content of the resin extracts of *C. mukul*, *G. resinifera* and *S. robusta*.

CONCLUSION

Present study revealed that the resin extracts of *Boswellia serrata*, *Commiphora mukul*, *Gardenia resinifera* and *Shorea robusta* possessed antioxidant properties and showed +ve correlation between the total antioxidant capacity and the total phenolic and flavonoid contents. Among all the extracts tested, the highest antioxidant capacity, reducing power activity and DPPH radical scavenging activity were shown by the extract of *G. resinifera*. There is a strong +ve correlation between the total antioxidant capacity of *G. resinifera* and the phenolic content.

The antioxidant components such as phenolics and flavonoids from the resin of *G. resinifera* may be isolated and identified in future to explore their potential as a source of new antioxidants.

ACKNOWLEDGEMENT

The authors express their sincere thanks to Principal, St. Xavier's college for providing the facilities. Thanks to Dr. Santhosh and Dr. Rashmi for their help in collection of gum resin.

CONFLICT OF INTEREST

There is no conflict of interest among authors of this publication.

REFERENCES

1. Sahu KG, Khadabadi SS, Bhide SS. Evaluation of In vitro antioxidant activity of *Amorphophallus campanulatus* (Roxb.) Ex Blume Decne. Int J Chem, Sci, 2009; 7(3): 1553-1562.
2. Sarmah P, Baishya D. Phytochemical analysis and antioxidant activity of *Gardenia jasminoides* Ellis and *Diospyros malabarica* Kostel. Int J Pharm Bio Sci, 2014; 5(1): 199-204.
3. Mondal SK, Chakraborty G, Gupta M, Mazumder UK. Hepatoprotective activity of *Diospyros malabarica* bark in carbon tetrachloride intoxicated rats. European Bull Drug Res, 2005; 13, 25.
4. Murthy TK. Minor forest produce used in pharmaceutical and other industries. Hyderabad: Pharma Med Press: 2010; pp. 221.
5. Nadkarni KM. The Indian Materia Medica. 3rd ed., Popular prakashan, Ahmedabad: 1976 Vol I pp. 791, 881.
6. Khare, CP. Indian medicinal plants: An illustrated dictionary. Springer, India pvt ltd. New Delhi: 2007.
7. Wani TA, Kumar D, Prasad R, Verma PK, Sardar KK, Tandan SK. Analgesic activity of the ethanolic extract of *Shorea robusta* resin in experimental animals. Indian J Pharmocology, 2012; 44(4): 493-9.
8. Joshi SG. Medicinal plants, Oxford and IBH publishing co pvt ltd New Delhi: 2000; pp. 109,110.
9. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal plants. Central Drug Research institute, Lucknow and publication and information Directorate; New Delhi: 1985-1989, pp. 215.

10. Bellamkonda R, Rasineni K, Singareddy SR, Kasetti RB, Passuria R, Chippada. Antihyperglycemic and antioxidant activities of alcoholic extract of *Commiphora mukul* gum resin in Streptozotocin induced diabetic rats. Int society for Pathophysiology, 2011; 18(4): 255-61.
11. Kirtikar KR and Basu BD. Indian Medicinal Plants. 2nd ed., Dehradun; International Book Distributors: 1987; pp. 1279.
12. Chopra RN, Nayar SL, Chopra LC. Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi: 1956; pp. 123.
13. Kumar ST, Shanmugam S, Palvannam T, Kumar Bharathi VM. Evaluation of antioxidant properties of *Canthium parviflorum* Lam. Leaves. Natural Product Radiance, 2008; 7(2): 122-126.
14. Peer BD, Ravishankar K, Kiranmayi GVN and Subbarao M. Antioxidant activity of different leaf extracts of *Ocimum sanctum*, *Mangifera indica* and *Hibiscus rosa sinesis.* Int. J. Pharm. Sci. Res, 2014; 25(2): 296-9.
15. Pillai SS and Mini S. In vitro antioxidant activities of different solvent fractions from the ethanolic extract of *Hibiscus rosa sinensis* petals. Int J Pharmaceutical sci and Research, 2014; 5(9): 3879-85.
16. Jayanthi P, Lalitha P. Reducing power of solvent extracts of *Eichhornia crassipes* (Mart.) Solms. Int J Pharmacy and Pharmaceutical sci, 2011; 3(3): 126-8.
17. Mahmoudi M, Ebrahimzadeh MA, Nabavi SF, Hafezi S, Nabavi SM, Eslami SH. Antiinflammatory and antioxidant activities of gum mastic. European Review for Medical and Pharmacological Sciences, 2010; 14: 765-9.
18. Motlhanka DMT, Habtmariam S, Houghton P. Free radical activity of crude extracts and 4'-O-Methylepigallocatechin isolated from roots of *Cassine transvaalensis* Burtt-Davy from Botswana. African J Biomedical Res, 2008; 11: 55-63.
19. Shanmugam S, Kumar ST, Selvam KP. Laboratory Handbook on Biochemistry, PHI Learning Private Limited; New Delhi: 2010; pp. 98-102.