Chapter 13

Antioxidant and antibacterial activities of *Garcinia* species in the Western Ghats

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Abstract

Garcinia species are reputed for the diversity of phenolic compounds such as biflavonoids, xanthones and benzophenones that can act as antioxidants. In the present study, various *in vitro* methods were used to investigate the antioxidant properties of nine *Garcinia* species in the Western Ghats. DPPH radical scavenging activity of *G. talbotii* was higher (IC_{50}: 2.8±0.6 µg/mL) compared to standard compound ascorbic acid (IC_{50}: 3.2±0.5 µg/mL), while *G. pushpangadaniana* showed the highest superoxide radical scavenging activity (IC_{50}:16.75±0.99µg/mL) and reducing activity. The potential antioxidant activities of the *Garcinia* species were in corroboration with the high phenolic and flavonoid contents present in these species. The antibacterial activities of the leaf methanol extracts were however negligible or nil, except against the Gram positive strain, *Bacillus subtilis.*

Keywords: Antioxidant, Antibacterial, *Garcinia* species, DPPH, Superoxide radical, Reducing power, *Bacillus subtilis*

Introduction

Oxygen is an indispensable element for life and is necessary for aerobic respiration in animals. However, reactive oxygen species (ROS) such as superoxide anion radicals (O_2^-), hydroxyl radicals (OH^-) and non-free radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen, that are continuously produced during the normal metabolism of oxygen, are harmful to biological systems. Healthy humans can detoxify or eliminate these free radicals by enzymes such as superoxide dismutase, catalase, and peroxidase (Gulcin, 2006; Terashima *et al*., 2010). If the oxidative damage is beyond the capacity of the natural repair mechanisms of the cells, it may trigger several chronic diseases (Franco, 2008).

The consumption of diets which are rich in antioxidants can protect the human body from oxidative stress and associated diseases induced by endogenous and exogenous factors (Morganti, 2009). These health effects have been partially attributed to the presence of phenolic compounds in plants (Guo *et al*., 2011). *Garcinia* species are known to be rich in phenolic compounds such as flavonoids, phenolic acids, xanthones, biflavonoids and benzophenones. There are many compounds reported from the genus *Garcinia* with higher free radical scavenging activities compared to known standards. Griffipavixanthone, a prenylated xanthone isolated from *Garcinia virgata* was reported to possess promising antioxidant activity with lower EC_{50} value compared to the references BHA and α-tocopherol

(Merza *et al*., 2004). The phloroglucinol parvifoliol E from *Garcinia parvifolia* showed remarkable antioxidant acivity compared to standard BHT (Rukachaisirikul *et al*., 2006). 1,3,5,7-Tetrahydroxyxanthone exhibited strong antioxidant activity comparable to the reference molecule probucol (Jantan *et al*., 2012). α-Mangostin is a common xanthone reported from different *Garcinia* species, that exhibited stronger antioxidant activity than α-tocopherol in ferric thiocyanate (FTC) assay (Taher *et al*., 2012). Biflavonoids are dimers of two flavonoids, limited in distribution to some genus. This interesting group of compounds was reported from different *Garcinia* species and many of them exhibited remarkable antioxidant activities. The flavanone-(3-8")-flavone biflavonoid morelloflavone displayed considerable antioxidant activity and was more potent than quercetin (Osorio *et al*., 2013). 1,3,6-trihydroxy-7-methoxy-2,8-(3-methyl-2-butenyl) xanthone isolated from *Garcinia hombroniana* exhibited stronger antioxidant activity than the standard compounds trolox, gallic acid and ascorbic acid (Jamila *et al*, 2014). *Garcina* species were reported to possess remarkable level of activities against different diseases and the antioxidant activities of phenolic compounds from the genus have a major role in the mechanism of bioactivities.

Recently, a wide range of plants have been screened for antimicrobial property, because of the increased microbial resistance and harmful side effects of existing antimicrobial agents (Djeussi *et al*., 2013). *Garcinia* species have also been a subject of antimicrobial screening and potential activities have been reported for extracts and isolated compounds from several *Garcinia* species (Negi *et al*., 2008; Policegoudra, 2012; Fouotsa *et al*., 2013; Semwal *et al*., 2015).

Although the *Garcinia* species are gaining much attention worldwide due to their potential bioactivities, the *Garcinia* species in the Western Ghats are least investigated for their bioactivities. The present chapter elaborates the antioxidant and antibacterial activities of the leaf methanol extracts of nine *Garcinia* species (*G. gummi-gutta, G. imberti, G. indica, G. Morella, G. pushpangadaniana, G. rubro-echinata, G. talbotii, G. travancorica and G. wightii*) from the Western Ghats.

1. *In vitro* antioxidant activity of *Garcinia* species in the Western Ghats

Antioxidants act by several mechanisms and it is difficult to predict the full spectrum of activity in a single assay. In the present study, *in vitro* methods such as DPPH scavenging assay, superoxide radical scavenging assay and reducing power assay were used to evaluate the antioxidant property of *Garcinia* leaf methanol extracts.

DPPH scavenging activity: Among free radical scavenging methods, DPPH method is more rapid, simple and inexpensive in comparison to other test models. DPPH (2, 2-diphenyl-1-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl) is a stable free radical that has an absorbance maximum in the visible region (517 nm). On accepting hydrogen from a donor, DPPH solutions lose the characteristic deep purple colour (Villano *et al*., 2007). The free radical scavenging activities of tested compounds are expressed as IC_{50} value, the concentration of the compound required to decrease the absorbance of DPPH solution by 50%.

Reducing power assay: In this method, antioxidant compound forms a coloured complex withpotassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700 nm. Increase in absorbance ofthe reaction mixture indicates the reducing power of the samples (Jayaprakash *et al*., 2008).

Superoxide radical scavenging assay: Superoxide anion radical is a weak oxidant that generates powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute significantly to oxidative stress. In the PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen and PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm thus indicates the consumption of superoxide anion in the reaction mixture. The superoxide anion scavenging activity was measured as described by Robak and Gryglewski (1988).

Total phenolic and flavonoid contents: Phenolic compounds consist of diverse group of secondary metabolites such as flavonoids, anthocyanins, coumarins, xanthones, benzophenones and phenolic acids, and possess ideal structural features for free radical scavenging activity. Antioxidative properties of phenolic compounds are due to different mechanisms such as scavenging of free radicals, chelation of metal ions like iron and copper, and inhibition of enzymes responsible for free radical generation (Benavente-Garcia, 1997; Rice-Evans *et al*., 1997). The phenol content was determined by Folin-Ciocateu reagent method (McDonald *et al*., 2001). The content of flavonoids was determined by aluminum chloride colourimetric method (Chang *et al*., 2002).

Leaf methanolic extrcacts of9 *Garcinia* species from the Western Ghats (*G. gummi-gutta, G. imberti, G. indica, G. morella, G. pushpangadaniana, G. rubro-echinata, G. talbotii, G. travancorica* and *G. wightii*) were subjected to antioxidant evaluation using different *in vitro* methods. Most of the species showed remarkable levels of antioxidant activities using *in vitro* models like DPPH radical scavenging assay, reducing power assay and super oxide radical scavenging assay (**Table 1**). Among the species studied *G. talbotii* (IC_{50}2.8±0.6 µg/mL), *G. rubro-echinata* (IC_{50}6.5±0.8 µg/mL), *G. imberti* (IC_{50}9.0±1.2 µg/mL), and *G. wighti* (IC_{50}16.0±2.0 µg/mL) showed a promising level of DPPH radical scavenging activity compared to standard ascorbic acid with IC_{50} value of 3.2±0.5 µg/mL. IC_{50} of *G. talbotii* leaf methanol extract against DPPH radical was higher than that of standard ascorbic acid.

Superoxide radical scavenging activity revealed a moderate level of activity compared to the standard ascorbic acid (IC_{50} value of 5.8±0.25 µg/mL). Among the species studied, *G. pushpangadaniana* showed highest activity with IC_{50} value of 16.75±0.99 µg/mL and *G. indica* showed the minimal level of activity with IC_{50} value of 196.96±14.16 µg/mL. Superoxide radical scavenging activity of the extracts were not correlated to the phenolic or flavonoid contents.

Table 1. Phenolic and flavonoid contents and antioxidant activities of *Garcinia* leaf extracts

Sl. No.	*Garcinia* species	Total phenolics (mg/g)	Total flavonoids (mg/g)	DPPH IC_{50} (µg/mL)	Superoxide IC_{50} (µg/mL)
1	*G. gummi-gutta*	97.45±7.28	17.2±2.83	128±2	86.2±2.62
2	*G. imberti*	273.6±9.6	108±7.82	9±1.2	40.3±1.12
3	*G. indica*	46.67±15.08	11.1±1.84	558.3±18.65	196.96±14.16
4	*G. morella*	177.57±18.86	53.8±5.37	104±3.35	86.5±7.92
5	*G. pushpangadaniana*	884.6±83.51	197.3±9.47	9.04±0.83	16.75±0.99
6	*G. rubro-echinata*	392.85±7.28	48.05±2.19	6.5±0.8	27.2±0.42
7	*G. talbotii*	342.9±5.80	55.56±2.31	2.8±0.6	30.4±1.13
8	*G. travancorica*	435.53±23.85	143.4±11.60	18.9±1.8	53.2±3.09
9	*G. wightii*	239.3±24.18	239.0±26.87	16±2	27.6±0.7
10	Ascorbic acid	-	-	3.2±0.5	5.8±0.25

Leaf methanolic extracts of the *Garcinia* species studied showed varying levels of activity in reducing power assays (**Table 2, Figure 1**). The *Garcinia* species that contain higher amount of phenolics, especially *G. pushpangadaniana*, *G. rubro-echinata* and *G. talbotii* showed remarkable activity in reducing power assay, whereas *G. gummi-gutta, G. indica and G. wightii* showed only moderate levels of activities.

Table 2. Reducing power assayof *Garcinia* species leaf extracts at different concentrations-Absorbance at 700 nm

Garcinia species	20 (µg/mL)	40 (µg/mL)	60 (µg/mL)	80 (µg/mL)	100 (µg/mL)
G. gummi-gutta	0.026	0.045	0.082	0.103	0.122
G. rubro-echinata	0.026	0.308	0.503	0.669	0.858
G. imberti	0.011	0.172	0.39	0.55	0.678
G. indica	0.034	0.054	0.068	0.08	0.09
G. morella	0.051	0.133	0.196	0.255	0.295
G. pushpangadani	0.231	0.45	0.623	0.833	1.083
G. talbotii	0.185	0.347	0.5	0.681	0.721
G. travancorica	0.094	0.209	0.301	0.408	0.526
G. wightii	0.018	0.034	0.117	0.239	0.303

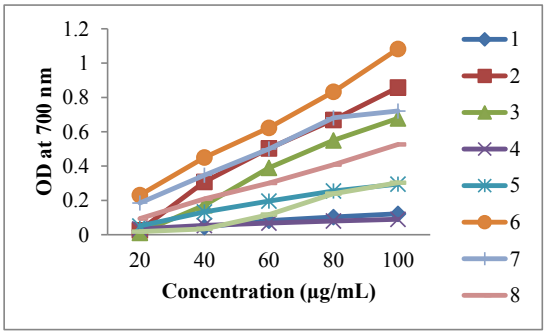

Figure 1. Reducing power assay of *Garcinia* leaf extracts (1- *G. gummi-gutta*, 2- *G. rubro-echinata*, 3- *G. imberti*, 4- *G. indica*, 5- *G. morella*, 6- *G. pushpangadaniana*, 7- *G. talbotii*, 8- *G. travancorica*, 9- *G. wightii*)

2. Antibacterial activity of *Garcinia* leaf methanol extracts

The plant extracts were dissolved in DMSO was used for the assay. The Kirby-Bauer method was used for antimicrobial susceptibility testing (Cappucino and Sherman1999). Briefly, the Mueller Hinton Broth (MHB) containing specific organisms were incubated at 37°C until it achieved the 0.5 McFarland standards (~1.5 x 10^8 CFU/ml). The dried surface of the Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. The discs impregnated with the extracts were placed on Mueller Hinton agar and incubated at 37^0 for 16-18 hours. After incubation, the diameters of the zones of complete inhibition were measured, including the diameter of the disc.

Table 3. Antibacterial activity (zone of inhibition in mm) of *Garcinia* leaf methanol extracts and standard kanamycin sulphate

Garcinia species	Conc. (µg/disc)	*P. vulgaris*	*E. faecalis*	*S. marscenes*	*P. aeruginosa*	*S. typhi*	*B. subtilis*	*S. mutants*
G. cowa	100	Nil	Nil	Nil	Nil	Nil	9.0	Nil
	500	Nil	Nil	Nil	Nil	Nil	9.5	Nil
	1000	Nil	Nil	Nil	Nil	Nil	10	Nil
G. rubro-echinata	100	Nil	Nil	Nil	Nil	Nil	6.5	Nil
	500	Nil	Nil	Nil	Nil	Nil	7.5	Nil
	1000	Nil	Nil	7.5	Nil	Nil	9.0	7.5
G. gummi-gutta	100	Nil	Nil	Nil	Nil	Nil	7.5	Nil
	500	Nil	Nil	Nil	Nil	Nil	8.0	Nil
	1000	Nil	Nil	Nil	Nil	Nil	8.5	Nil
	500	Nil	Nil	Nil	Nil	Nil	9.5	7.0
	1000	Nil	Nil	Nil	Nil	Nil	10.5	8.0
G. imberti	100	Nil	Nil	Nil	Nil	Nil	7.0	Nil
	500	Nil	Nil	Nil	Nil	Nil	9.0	Nil
	1000	Nil	Nil	Nil	Nil	Nil	10.5	Nil
G. indica	100	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	500	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	1000	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	500	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	1000	Nil	Nil	Nil	Nil	Nil	Nil	Nil
G. morella	100	Nil	Nil	Nil	Nil	Nil	6.5	Nil
	500	Nil	Nil	Nil	Nil	Nil	8.0	7.0
	1000	Nil	Nil	Nil	Nil	Nil	9.5	8.0
G. pushpangadaniana	100	Nil	Nil	Nil	Nil	Nil	7.0	6.5
	500	Nil	Nil	Nil	Nil	Nil	9.5	7.5
	1000	Nil	Nil	Nil	Nil	Nil	12.5	10.0
G. talbotii	100	Nil	Nil	Nil	Nil	Nil	10	9.0
	500	Nil	Nil	Nil	Nil	Nil	12	10.0
	1000	Nil	Nil	Nil	Nil	Nil	13.5	13.0
G. travancorica	100	Nil	Nil	Nil	Nil	Nil	8.0	Nil
	500	Nil	Nil	Nil	Nil	Nil	10.0	Nil
	1000	Nil	Nil	Nil	Nil	Nil	11.0	Nil
G. wightii	100	Nil	Nil	Nil	Nil	Nil	9.0	Nil
	500	Nil	Nil	Nil	Nil	Nil	11.0	6.5
	1000	Nil	Nil	Nil	Nil	Nil	12.0	8.0
	500	Nil	Nil	Nil	Nil	Nil	9.0	Nil
	1000	Nil	Nil	Nil	Nil	Nil	10.0	9.0
Kanamycin sulphate	30	20.0	22.0	25.0	20.0	27.0	28.0	25.0

In most of the cases, the extracts were inactive against the tested strains of bacteria (**Table 3**). Remarkable observation was the moderate activity against the gram positive *Bacillus subtilis* for all the extracts except *G. indica.* It is interesting to note that previous reports also reveal the activity of *Garcinia* extracts and compounds against Gram positive strains, especially *Bacillus subtilis* (Rao and Natarajan, 1950, Negi *et al*., 2008; Semwal *et al*., 2015).

The antimicrobial activities of *Garcinia* leaf methanol extracts against food pathogens such as *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Salmonella enteric* ser.*typhi,* and *Vibrio cholera* were also screened (**Table 4**). The MIC values were determined by modified broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) (2009). Briefly, 200µl of Mueller Hinton Broth (MHB) was placed into each to wells of 96 well microplate. The plant extracts were dissolved in DMSO, and diluted to the required concentration. 1% of bacterial cell suspension was inoculated in MHB containing plant extracts and incubated at 37^0 C for 16 hours. *Garcinia* leaf methanol extracts were active against the Gram positive strains screened; *Bacillus cereus* and *Staphylococcus aureus*.

Table 4. Antibacterial activity (MIC in µg/ml) of *Garcinia* leaf methanol extracts against food pathogens

Garcinia species	*Escherichia coli* MTCC 441	*Bacillus cereus* MTCC430	*Staphylococcus aureus* MTCC7443	*Salmonella enterica* ser. *typhi* MTCC733	*Vibrio cholera* MTCC *3906*
G. pushpangadhania	Nil	100µg/ml	Nil	Nil	Nil
G. rubro-echinata	Nil	100µg/ml	100µg/ml	Nil	Nil
G. imberti	Nil	Nil	Nil	Nil	Nil
G. travancorica	Nil	Nil	Nil	Nil	Nil
G. talboti	Nil	Nil	Nil	Nil	Nil
G. morella	Nil	200µg/ml	500µg/ml	Nil	Nil
G.wightii	Nil	100µg/ml	200µg/ml	Nil	Nil
G. gummi-gutta	Nil	Nil	Nil	Nil	Nil

Conclusions

Leaf methanol extracts of nine *Garcinia* species from the Western Ghats exhibited remarkable *in vitro* antioxidant activity against various free radicals. The potential antioxidant activities were in corroboration with the high phenolic and flavonoid contents. Antioxidant activity is directly correlated to several curing mechanisms and the present study highlights the potential of *Garcinia* species as targets for future drug development. However, the antibacterial activities of the leaf methanol extracts were nil or negligible against the tested strains, except for *Bacillus subtilis.*

References

1. Benavente-Garcia O, Castillo J, Marin FR, Ortuño A and Del Río JA. **1997**. Uses and properties of citrus flavonoids. *J. Agric. Food Chem.*, 45(12), 4505-4515.
2. Cappucino JG and Sherman N. **1999**. *Microbiology: A Laboratory Manual*, 5th edition. p.254, Benjamin Cumming Science Publishing, California.

3. Chang CC, Yang MH, Wen HM and Chern JC. **2002**. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.*, 10(3). 178-182.
4. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Ninth Edition. CLSI document M07-A9. **2009**. Clinical and Laboratory Standards Institute, Pennsylvania USA.
5. Djeussi DE, Noumedem JAK, Seukep JA, Fankam AG, Voukeng IK, Tankeo SB, Nkuete AHL and Kuete V. **2013**. Antibacterial activities of selected edible plants extracts against multidrugresistant gram-negative bacteria. *BMC Compl. Alt. Med.,* 13, 164.
6. Fouotsa H, Mbaveng A T , Mbazoa C D , Nkengfack A E , Farzana S , Iqbal C M , Meyer JJM, Lall N and Kuete V. **2013**. Antibacterial constituents of three Cameroonian medicinal plants: *Garcinia nobilis, Oricia suaveolens* and *Balsamocitrus camerunensis. BMC Compl. Alt. Med.,*13, 81.
7. Franco R, Schoneveld O, Georgakilas AG and Panayiotidis MI. **2008**. Oxidative stress, DNA methylation and carcinogenesis. *Cancer Lett.*, 266(1), 6-11.
8. Gulcin I. **2006**. Antioxidant and antiradical activities of L-carnitine. *Life Sci.*, 78(8), 803-811.
9. Guo T, Wei L, Sun J, Hou CL and Fan L. **2011**. Antioxidant activities of extract and fractions from *Tuber indicum* Cooke & Massee. *Food Chem.*, 127(4), 1634-1640.
10. Jamila N, Khairuddean M, Khan SN and Khan N. **2014**. Complete NMR assignments of bioactive rotameric (3→8) biflavonoids from the bark of *Garcinia hombroniana*. *Magnetic Res. Chem.*, 52(7), 345-352.
11. Jantan I and Saputri FC. **2012**. Benzophenones and xanthones from *Garcinia cantleyana* var. cantleyana and their inhibitory activities on human low-density lipoprotein oxidation and platelet aggregation. *Phytochemistry*, 80, 58-63.
12. Jayaprakasha GK, Girennavar B and Patil BS. **2008**. Radical scavenging activities of Rio Red grapefruits and Sour orange fruit extracts in different in vitro model systems. *Bioresource Tech.*, 99(10), 4484-4494.
13. McDonald S, Prenzler PD, Antolovich M and Robards K. **2001**. Phenolic content and antioxidant activity of olive extracts. *Food Chem.*, 73(1), 73-84.
14. Merza J, Aumond MC, Rondeau D, Dumontet V, Le Ray AM, Séraphin D and Richomme P. **2004**. Prenylated xanthones and tocotrienols from *Garcinia virgata*. *Phytochemistry*, 65(21), 2915-2920.
15. Morganti P. **2009**. The photoprotective activity of nutraceuticals. *Clin. Dermatol.*, 27(2), 166-174.
16. Negi PS, Jayaprakasha GK and Jena BS. **2008.** Antibacterial activity of the extracts from the fruit rinds of*Garcinia cowa* and *Garcinia pedunculata* against food borne pathogens and spoilage bacteria. *Food Sc. Tech*., 41, (10), 1857-1861.
17. Osorio E, Londono J and Bastida, J. **2013**. Low-density lipoprotein (LDL)-antioxidant biflavonoids from *Garcinia madruno*. *Molecules*, 18(5), 6092-6100.
18. Policegoudra RS, Saikia S, Das J, Chattopadhyay P, Singh L and Veer V. **2012.** Phenolic content, antioxidant activity, antibacterial activity and phytochemical composition of *Garcinia lancifolia. Indian J. Pharm. Sci*., 74(3), 268-271.

19. Rao RR and Natarajan S. **1950**. On morellin, the antibacterial principle of the seeds of *Garcinia morella* Desrous. *Curr. Sci.,*19 (02) 59-60.
20. Rice-Evans C, Miller N and Paganga G. **1997**. Antioxidant properties of phenolic compounds. *Trends Plant Sci.*, 2(4), 152-159.
21. Robak J and Gryglewski RJ. **1988**. Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol*., 37, 837-841.
22. Rukachaisirikul V, Naklue W, Phongpaichit S, Towatana NH and Maneenoon K. **2006**. Phloroglucinols, depsidones and xanthones from the twigs of *Garcinia parvifolia*. *Tetrahedron*, 62(36), 8578-8585.
23. Semwal RB, Semwal DK, Vermaak I and Viljoen A. **2015**. A comprehensive scientific overview of *Garcinia cambogia. Fitoterapia,* 102, 134-148.
24. Taher M, Susanti D, Rezali MF, Zohri FSA, Ichwan SJA, Alkhamaiseh SI and Ahmad F. **2012**. Apoptosis, antimicrobial and antioxidant activities of phytochemicals from *Garcinia malaccensis* Hk. f. *Asian Pacific J. Trop. Med.*, 5(2), 136-141.
25. Terashima M, Watanabe R, Ueki M and Matsumura S. **2010**. Comprehensive evaluation of antioxidant activity for various substances with 5-axe cobweb chart. *Food Chem.*, 120(1), 150-155.
26. Villano D, Fernandez-Pachon MS, Moya ML, Troncoso AM and Garcia-Parrilla MC. **2007**. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. *Talanta*, 71(1), 230-235.