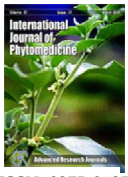
International Journal of phytomedicine 4 (2012) 304-409
http://www.arjournals.org/index.php/ijpm/index



ISSN: 0975-0185

Short Communication

Phytochemical constituents and antioxidant activity of various fractions of *Guazuma tomentosa* root heartwood

Renuka Jain*[1], Namita Yadav[1]

*Corresponding author:

Renuka Jain

[1]Department of Chemistry, University of Rajasthan, Rajasthan, Jaipur-302004

Abstract

Guazuma tomentosa is an important medicinal plant. The present investigation deals with GC-MS analysis of pet.ether, dichloromethane and ethyl acetate fractions of root heartwood of *G. tomentosa*. In antioxidant activity of these fractions by employing DPPH free radical scavenging effect and FRAP total reduction capability method dichloromethane fraction was most effective exhibiting activity nearly equivalent to that of ascorbic acid (standard) at higher concentration, which could be attributed to the phenolic constituents in this fraction. Results indicated that dichloromethane fraction can be a potential source of natural antioxidant agents.

Keywords: GC-MS, Guazuma tomentosa, DPPH, FRAP, antioxidant.

Introduction

Guazuma tomentosa Kunth. syn. G. *ulmifolia* Lamk. (commonly known as "guacimo" or "mutamba") is a middle-sized tree, belonging to the family Sterculiaceae, which occurs naturally throughout Latin America [1]. In India only this species is grown out of the existing five.

In popular medicine, *G. tomentosa* is traditionally used in several countries to treat bronchitis, burns, diarrhea, asthma, inflammations and alopecia. Its bark is used in the treatment of diarrhea, hemorrhages, fever, chest diseases [2,3], gastrointestinal pain, hypertension and as stimulant for uterine contractions [4].

Previous investigations of the chemical composition of *G. tomentosa* have indicated the occurrence of procyanidins, cyanogenic glycosides, triterpenes, diterpenes, sesquiterpenes, flavonoids, coumarins and condensed tannins from bark [5-7], heartwood [8,9], leaves [10-12], flowers [13] and roots [14].

Antioxidants are compounds that protect cells against the damaging effect of reactive oxygen species such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrile by inhibiting or quenching the free radicals. There is a consensus of opinion that free radical induce oxidative damage to biomolecules. It is now well established that a series of oxygen-centred free radicals and other reactive oxygen species (ROS) contribute to the pathology of many disorders including atherogenesis, neurodegeneration, chronic inflammation, cancer and physiological senescence [15]. Therefore, antioxidants are considered important nutraceuticals on account of their many health benefits and they are widely used in the food industry as potential inhibitors of lipid peroxidation [16]. Antioxidants scavange free radicals by initiating and propagating oxidative chain reactions, and thus can delay or prevent intracellular oxidative damage [17]. Several methods have been proposed to measure the antioxidant activity of pure compounds and plant extracts, such as FRAP (Ferric Reducing Antioxidant Power), ORAC (Oxygen Radical Absorbance Capacity), ESR (Electron Spin Resonance), ABTS (2,2-azinobis(3-ethyl-benzothiazoline-6-sulphonate) and DPPH (2,2-diphenyl-1-picrylhydrazyl). Out of these methods we have carried antioxidant activity by employing DPPH and FRAP method. The objective of this study was to evaluate the phytochemicals using GC-MS analysis and evaluating their antioxidant activity.

Materials and Methods

Reagents and instrument

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and standard ascorbic acid was purchased from Sigma (USA). Methanol and all other solvents were of analytical grade (Merck).

Absorbance measurements were made using SCHIMADZU-1800 UV spectrophotometer.

Collection of Plant material

The roots of *Guazuma tomentosa* were collected from the University of Rajasthan Campus, Jaipur, Rajasthan, India in September, 2010 during daytime. The plant was authenticated at the Herbarium of the Department of Botany, University of Rajasthan, Jaipur (Herbarium Sheet No. RUBL 19762).

Plant extraction

The root heartwood was shade dried, exhaustively extracted with ethanol (3x8 hrs) and evaporated to dryness using a rotary evaporator. The residue was fractioned with pet.ether, dichloromethane and ethyl acetate successively. Fractions were collected, evaporated to dryness using a rotary evaporator.

Gas chromatography-mass spectrometry (GC-MS) analysis

Preparation of Plant Extract

The fractions collected were dissolved in chloroform and the contents were filtered through Whatman No. 1 paper (Merck, Mumbai, India) to remove particulate matter. The samples were then subjected to analysis.

Chromatographic conditions

The GC-MS analysis was performed with a Shimadzu GC-MS-QP 2010 Plus using a RTX-5 (60m x 0.25mm x 0.µm) capillary column with 5% diphenyl, 95% dimethyl polysiloxane stationary phase. Column temperature was 100º initially, held for 2 min, then programmed to 200ºC at a rate of 15ºC / min and held for 5 min; finally programmed to 300ºC at a rate 20ºC/min, then held for 27 min, run time 40 min. The sample volume injected was 0.4µl with splitless mode and pressure at column inlet was 169.6 kPa with helium (flow rate of 0.7ml/min.) as a carrier gas. The ion source was set at 250º and the method of electron-impact ionisation was applied. All data were obtained by collecting the full scan mass spectra within the scan range 40 to 950 amu.
Compounds were identified by comparison of mass spectra with those in the Wiley and NIST libraries and mass spectra of standards.

DPPH free radical-scavenging effect

DPPH assay was carried out according to the method Khalaf *et al.* [18] A solution (2.5 ml) of $2x10^{-3}$ µg/ml of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol was mixed with equal volume of extract/test compound/ascorbic acid (standard) solution in methanol and kept in dark for 30 min. The absorbance at 517 nm was monitored at different concentrations (10, 20, 40, 60, 80 µg/ml) using UV-Vis spectrophotometer. Blank was also carried out to determine the absorbance of DPPH, before interacting with the extract. The absorbance was measured and % inhibition was calculated using the formula.

Percent (%) inhibition of DPPH activity = [A-B/A] x 100
Where A is the absorbance of the blank and A is the absorbance in the presence of test compound.

FRAP total reduction capability effect

Fe^{3+} - Fe^{2+} transformation assay was carried out following the method of Oyalzu [19]. To 1 ml of extract/test compound/ascorbic acid (standard) at different concentrations (62.5, 125, 250, 500, 1000 µg/ml) in ethanol was added 1 ml of distilled water, 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixture was incubated at 50ºC for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture, which was then centrifuged for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm using UV-Vis spectrophotometer. Higher absorbance indicates greater reducing power.

Results and Discussion

GC-MS analysis revealed the presence of 11 long chain compounds, 4 aromatic components, 3 steroids, 1 phenolic component, 1 triterpenoid and 1 coumarin in the pet.ether fraction (Table 1). Dichloromethane fraction consists of 14 long chain compounds, 2 isoflavones, 6 phenolic components, 2 terpenoid, 1 aromatic component, 1 flavonol glycoside and 1 isocoumarin (Table 2) while the ethyl acetate fraction comprises of 14 long chain compounds, 2 aromatic component, 3 phenolic compounds, 1 coumarin and 1 napthaquinone (Table 3).
The radical scavenging activity of pet.ether, dichloromethane and ethyl acetate fractions was determined by the reduction in absorbance at 517 nm due to scavenging of stable DPPH free radical. The positive DPPH test suggests that the samples are free radical scavengers. The scavenging effects of pet.ether, dichloromethane and ethyl acetate fractions on the DPPH radical are illustrated and compared in Table 4. Dichloromethane fraction had significant scavenging effects on the DPPH radical at higher concentration.
It is reported that the antioxidant activity of plants is closely associated with their reducing power, hence it was evaluated using the FRAP method. FRAP is a simple and speedy method that actually measures the reducing capability of antioxidants and screens for the ability to maintain the redox status in cells. Our present results indicated that dichloromethane fraction indeed has the highest reducing power at higher concentration (Table 5), which is consistent with the free radical-scavenging capacity observed in the DPPH scavenging activity.

Table 1. Components of pet.ether fraction

Peak#	R.Time	Area	Area%	Name
1	8.242	280666	0.65	Nonanoic acid
2	9.156	1313392	3.02	Decanoic acid
3	10.529	276163	0.63	Unidentified
4	10.945	2648424	6.09	1,2,3-Trimethoxy-5-(2-propenyl) benzene
5	11.197	645252	1.48	Pentadec-1-ene
6	11.645	2505642	5.76	2,6-dimethoxy-4-(2-propenyl) phenol
7	14.014	533694	1.23	Hexadec-1-ene
8	14.110	977560	2.25	Octadecane
9	14.845	720588	1.66	6,10-Dimethylundecanoate
10	15.325	1650390	3.79	Bis (2-methylpropyl) benzene-1,2-dicarboxylate
11	15.843	2210867	5.08	Methyl hexadeconate
12	16.448	3278054	7.54	Di butyl benzene-1,2-dicarboxylate
13	16.567	6579816	15.13	Octadec-9-enoic acid
14	16.633	1925071	4.43	Octadecane
15	17.468	2028138	4.66	3,4,7-trimethoxy coumarin
16	17.640	580858	1.34	7,10-Hexadecadienoic acid
17	18.426	562992	1.29	n-triacontane
18	19.692	581924	1.34	Unidentified
19	20.947	8538474	19.63	Bis (2-ethylhexyl) benzene-1,2-dicarboxylate
20	26.563	285880	0.66	β-Sitosterol 3-acetate
21	32.073	336683	0.77	4,2,2-Stigmastadiene-3-one
22	33.632	3706799	8.52	Stigmast-4-ene-3-one
23	36.585	311911	0.72	Silicon oil
24	39.741	1012435	2.33	Methyl (2β,3β)-2,3,23-trihydroxyolean-12-en-28-oate
		43491673	100.00	

Table 2. Components of dichloromethane fraction

Peak#	R.Time	Area	Area%	Name
1	8.101	1615561	0.35	Unidentified
2	9.263	49536505	10.64	Tetradecane
3	9.825	1555858	0.33	(1-Methyl heptyl) cyclohexane
4	10.078	2600543	0.56	1-Dodecene
5	10.210	2566995	0.55	n-Pentadecane
6	10.549	13234593	2.84	Unidentified
7	10.965	3114173	0.67	Unidentified oxidation product
8	11.381	105033958	22.56	n-Heptadecane
9	11.670	8967694	1.93	Ferulic acid
10	11.945	5473449	1.18	3-O-Galloyl quinic acid butyl ester
11	12.159	1481851	0.32	Tridecylcyclohexane
12	12.287	1440507	0.31	Pentadecanone
13	12.535	8970825	1.93	Tetradecyl acrylate
14	14.132	38225132	8.21	Fomononetin
15	14.248	58683867	14.248	Octadecane
16	15.261	2925857	15.261	8-Octadecanone
17	15.347	3086531	15.347	Methyl adamantine ethanoic acid
18	16.468	11386148	2.45	8-Pentadecanone
19	16.713	59263111	12.73	Eicosane
20	17.472	2012375	17.472	3-[2'-(4"-Methoxy phenyl)ethyl]isocoumarin
21	17.954	2053099	0.44	Quercetin
22	18.208	2276046	0.49	Santolina alcohol
23	18.468	31090980	6.68	Feruloyl glucose
24	19.832	17559553	3.77	6"-Malonyldaidzin
25	20.485	1266911	0.27	β-Caryophyllene
26	20.943	7769243	1.67	Bis(2-ethyl hexyl) benzene-1,2-dicarboxylate
27	21.113	9739356	2.09	Trans-p-ferulyl alcohol-4-O-(6'-2 methyl-3 hydroxypropionyl)glucopyranoside
28	22.527	4358616	0.94	Dotriacontane
29	24.311	1267070	0.27	Unidentified
30	31.434	3637990	0.78	Eicosanoyl-3-O-feruloyl quinate
31	36.788	3456344	0.74	Epimedoside A
		465650741	100.00	

Table 3. Components of ethyl acetate fraction

Peak#	R.Time	Area	Area%	Name
1	7.469	1736336	5.00	Dimethyl 2-propoxybutanedioate
2	10.790	807824	2.33	Dimethyl nonadioate
3	10.953	884999	2.55	5-Allyl-1,2,3-trimethoxy benzene
4	11.201	1088950	3.14	Tetradec-1-ene
5	11.275	1793385	5.16	Heptadecane
6	11.677	3890203	11.20	4-Allyl syringol
7	11.981	518628	1.49	3,4,5-Trimethoxy phenol
8	12.511	1122146	3.23	2-Propenoic acid
9	14.024	404740	1.17	Hexadec-1-ene
10	14.123	876545	2.52	Eicosane
11	14.259	1788439	5.15	Gallic acid trimethyl ether
12	14.800	2435170	7.01	Unidentified
13	15.872	5139648	14.80	Methyl hexadecanoate
14	16.454	510025	1.47	Octadecanoic acid
15	16.608	1502335	4.33	Ethyl hexadecanoate
16	17.528	2414524	6.95	3,4,7-Trimethoxy coumarin
17	17.656	2596557	7.48	Methyl octa deca-9,12-dienoate
18	17.880	282488	0.81	Tetracosanoic acid
19	18.018	3278237	9.44	5,8-Dihydroxy-2,7-dimethoxy-1,4-napthalenedione
20	18.203	463048	1.33	9,12-Octadecadienoic acid
21	18.396	722727	2.08	1-Tetracosanol
22	19.807	265537	0.76	Unidentified
23	20.931	203298	0.59	Dinonyl benzene-1,2-dicarboxylate
		34725789	100.00	

Table 4. Antioxidant activity of root heartwood fractions of *G. tomentosa* by DPPH method

Nature of extract	% Inhibition (concentration in µg/ml)				
	10	20	40	60	80
Pet.ether	39.97 ± 0.31	53.05 ± 0.25	60.15 ± 0.17	64.47 ± 0.17	66.25 ± 0.12
Dichloromethane	63.80 ± 0.25	81.32 ± 0.06	85.05 ± 0.26	92.95 ± 0.21	93.85 ± 0.07
Ethyl acetate	55.67 ± 0.16	64.30 ± 0.09	73.10 ± 0.11	75.30 ± 0.14	80.82 ± 0.10
Ascorbic acid	93.7	94.3	96.25	97.02	97.2

Table 5. Antioxidant activity of root heartwood fractions of *G. tomentosa* by FRAP method

Nature of extract	Absorbance				
	62.5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Pet.ether	0.159 ± 0.01	0.242 ± 0.41	0.283 ± 0.02	0.324 ± 0.01	0.370 ± 0.02
Dichloromethane	0.407 ± 0.01	0.528 ± 0.03	0.635 ± 0.01	0.960 ± 0.05	1.211 ± 0.11
Ethyl acetate	0.381 ± 0.03	0.412 ± 0.01	0.418 ± 0.03	0.499 ± 0.14	0.627 ± 0.01
Ascorbic acid	0.553	0.813	1.052	1.257	1.308

Conclusion

Among the fractions tested, dichloromethane fraction demonstrated the best effect nearly equivalent to that of ascorbic acid (standard) at higher concentration. This antioxidant activity of dichloromethane fraction might be attributed to the phenolic constituents such as flavonoids, isocoumarins and phenolic acid derivatives which is in conformity with the earlier reports [20,21]. Ethyl acetate fraction exhibited moderate activity while pet.ether fraction exhibited trace activity at all concentrations.

Acknowledgements

The authors are grateful to the AIRF, Jawahar Lal Nehru University, New Delhi for the GC-MS facility and to CSIR, New Delhi for providing financial assistance.

References

[1]. Barbosa, JM, Macedo, AC. Essências Florestais Nativas de Ocorrência no Estado de São Paulo: Informações Técnicas sobre Sementes, Grupos Ecológicos, Fenologia e Produção de Mudas. Instituto de Botânica e Fundação Florestal, São Paulo, SP, Brazil: 1993. p. 125.

[2]. Kirtikar KR, Basu BD. Indian Medicinal Plants. Lalit Mohan Basu and Co. vol. 1. Allahabad: 1975. p. 381.

[3]. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. CSIR, New Delhi: 1971. p. 128.

[4]. Caballero-George C, Vanderheyden PM, Solis PN, Pieters L, Shaha AA, Gupta MP, Vauquelin G, Vlietinck AJ. Biological screening of selected medicinal Panamanian plants by radioligand-binding techniques. Phytomed. 2001; 8(1): 59-70.

[5]. Lopes GC, Bruschi ML, Mello JCP. RP-LC-UV Determination of proanthocyanidins in *Guazuma ulmifolia*. Chromatographia. 2009; 69(2): S175-S181.

[6]. Hoer M, Heinrich M, Rimpler H. Proanthocyanidin polymers with antisecretory activity and proanthocyanidin oligomers from *Guazuma ulmifolia* bark. Phytochem. 1996; 42(1): 109-119.

[7]. Lopes GC, Rocha JCB, de Almeida GC, de Mello JCP. Condensed tannins from the bark of *Guazuma ulmifolia* Lam. (Sterculiaceae). J. Brazilian Chem. Soc. 2009; 20(6): 1103-1109.

[8]. Prakash L, Garg G. Chemical constituents from the stem heartwood of *Guazuma tomentosa* Kunth (DC) and stem of *Clerodendron indicum* (Linn). J. Indian Chem. Soc. 1981; 58(7): 726-727.

[9]. Anjaneyulu ASR, Murty VS. Chemical examination of heartwood of *Guazuma tomentosa*. Indian J. Chem. 1981; 20B(1): 85-87.

[10]. Arriaga AM, Machado MIL, Craveiro AA, Pouliquen IBM. Oil analysis of *Guazuma ulmifolia* leaves. Rev. Bras. Farm. 1996; 77(2): 45-46.

[11]. Arriaga AMC, Machado MIL, Craveiro AA, Pouliquen YBM, Mesquita AG. Volatile constituents from leaves of *Guazuma ulmifolia* Lam. J. Essent. Oil Res. 1997; 9(6): 705-706.

[12]. Seigler DS, Pauli GF, Froehlich R, Wegelius E, Nahrstedt A, Glander KE, Ebinger JE. (Cyanogenic glycosides and menisdaurin from *Guazuma ulmifolia*, *Ostrya virginiana*, *Tiquilia plicata* and *Tiquilia canescens*. Phytochem. 2005; 66(13): 1567-1580.

[13]. Subramanian S, Sankara S, Marayana M. Flavonoids of the flowers of *Guazuma tomentosa* and *Delonix elata*. Curr. Sci. 1963; 32(7): 308-310.

[14]. Agarwal OP, Saxena U, Jain SC, Jain R. A new biscoumarin and other constituents from *Guazuma tomentosa* roots. Chem. Nat. Compd. 2010; 46(5): 713-715.

[15]. Ani V, Varadaraj MC, Naidu KA. Antioxidant and antibacterial activities of polyphenolic compounds from bitter cumin (*Cuminum nigrum* L.). Eur. Food Res. Tech. 2006; 224(1): 109–115.

[16]. Scherer R, Godoy HT. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chem. 2009; 112(13): 654–658.

[17]. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser. J. Free radicals and antioxidants in normal physiological functions and human disease. The Int. J. Biochem. Cell Biol. 2007; 39(1): 44–84.

[18]. Khalaf NA, Shakya AK, Al-Othman A, El-Agbar Z, Farah H, Antioxidant activity of some common plants. Turk. J. Biol. 2008; 32: 51-55.

[19]. Oyalzu M. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 1986; 44(6): 307-315.

[20]. Singh R, Singh S, Kumar S, Arora S. Studies on antioxidant potential of methanol extract/fractions of *Acacia auriculiformis* A. Cunn. Food Chem. 2007; 103(2): 505–511.

[21]. Aaby K, Hvattum E, Skrede G. Analysis of flavonoids andother phenolic compounds using high-performance liquid chromatography with coulometric array detection: Relationship to antioxidant activity. J. Agricul. Food Chem. 2004; 52(15): 4595–4603.