Int J Pharm Bio Sci 2013 Jan; 4(1): (P) 520 - 533

Research Article **Natural chemistry**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

AN EFFICIENT AND SIMPLE METHODOLOGY COUPLING MICROWAVE ASSISTED EXTRACTION TO GC-MS FOR THE IDENTIFICATION OF COMPONENTS IN ROOT BARK OF *GUAZUMA TOMENTOSA*

R. JAIN*, T. BHAGCHANDANI AND N. YADAV

Department of Chemistry, University of Rajasthan, Jaipur, India.

ABSTRACT

Microwave Assisted Extraction (MAE) of phytochemicals from medicinal plants has generated tremendous research interest and shown great potential. In this study, MAE method coupled with gas chromatography-mass spectrometry was used to monitor the organic compounds of root bark of *Guazuma tomentosa.* The extraction solvent, time and power were optimised prior to this. The GC-MS analysis of four fractions of the pet. ether extract led to the identification of 92 compounds including 17 flavonoids, 11 terpenoids, 3 steroids and a coumarin.

KEYWORDS: Microwave assisted extraction, *Guazuma tomentosa*, GC-MS analysis, flavonoids, terpenoids, coumarin.

R. JAIN
Department of Chemistry, University of Rajasthan, Jaipur, India.

Corresponding author

INTRODUCTION

Guazuma tomentosa Kunth. syn. *G. ulmifolia* Lamk., (Family: Sterculiaceae), belongs to a genus of five species, out of which only one is found in India. In traditional medicine, the inner bark of *G. tomentosa* is used for the treatment of elephantiasis and the infusion of old bark is considered sudorific and is useful in cutaneous and chest diseases [1, 2]. The plant is also known to have anti-diabetic [3], hypotensive and vasorelaxant [4], antiulcer [5], antibacterial [6] and antiviral [7] activities. Previous investigations on this plant indicated the presence of procyanidins, cyanogenic glycosides, terpenoids, flavanoids, coumarins and condensed tannins from bark [8], roots [9], stem bark [10], leaves [11,12], heartwood [13] and flowers[14]. All these investigations have employed conventional techniques like soxhlet, maceration, reflux and hydrodistillation for the purpose of extraction. These conventional methods suffer from severe drawbacks such as long extraction time, low efficiency, decomposition of thermolabile constituents etc. The use of large volumes of organic solvent associated with conventional methods is detrimental to environment and their subsequent disposal also becomes an issue of concern. Keeping in pace with such requirements recent times has witnessed the use and growth of new extraction techniques like microwave assisted extraction (MAE), supercritical fluid extraction (SCFE), pressurized solvent extraction (PSE) and ultrasound assisted extraction (UAE). Among these, MAE has been considered as a potential alternative to traditional extraction techniques. Many reports on the beneficial effects of MAE with respect to medicinal plants have been published [15, 16]. It has several potential advantages over the traditional methods, such as reduction of extraction time, reduced solvent usage, improved extraction yield, better accuracy and precision due to automation and is suitable for thermolabile constituents. We can even extract minute traces of constituents from a few milligram of plant sample using this method. It also provides agitation during extraction, which improves the mass transfer phenomenon. Until now, the extraction of *Guazuma tomentosa* with MAE method alongwith its GC-MS analysis is not reported. The purpose of the study is to develop a novel, eco-friendly and rapid MAE method for the efficient extraction of phytoconstituents from *Guazuma tomentosa*.

EXPERIMENTAL

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).

Extraction

For MAE, air dried and finely powdered root bark (1 gm) were mixed with 30 mL ethanol. After allowing a preleaching time of 5 min, the suspension was irradiated in a microwave oven (Samsung QW71X) for 9 min. The extraction process was performed in an intermittent way, i.e., irradiation:cooling:irradiation (one minute irradiation and one minute cooling). After extraction, the sample was centrifuged at 4000 rpm and the supernatant liquid was concentrated using rotary evaporator when a dark brown semi solid (0.0816 gm) was obtained. This was re-extracted with pet. ether at room temperature and chromatographed over a column of silica gel (60-120 mesh). Elution with solvents of increasing polarity afforded four fractions, viz. Fraction 1 (pet. ether), Fraction 2 (pet. ether : EtOAc = 7 : 3, v/v), Fraction 3 (pet. ether : EtOAc = 6 : 4, v/v), Fraction 4 (EtOAc : MeOH = 9 : 1, v/v) which were analysed by GC-MS.

GC-MS analysis

The GC-MS analysis was performed with a Shimadzu GC-MS-QP 2010 Plus fitted with a RTX-5 (60m x 0.25mm x 0.25µm) capillary

column. The carrier gas used was helium with a flow rate of 0.7 mL/min. The oven temperature was programmed from 100ºC (isothermal for 2 min), with an increase of 15ºC/min, to 200ºC (isothermal for 5 min), then 20ºC /min to 300ºC, ending with a 27 min isothermal at 300ºC. Total GC running time is 40 min. The ion source was set at 250ºC 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.

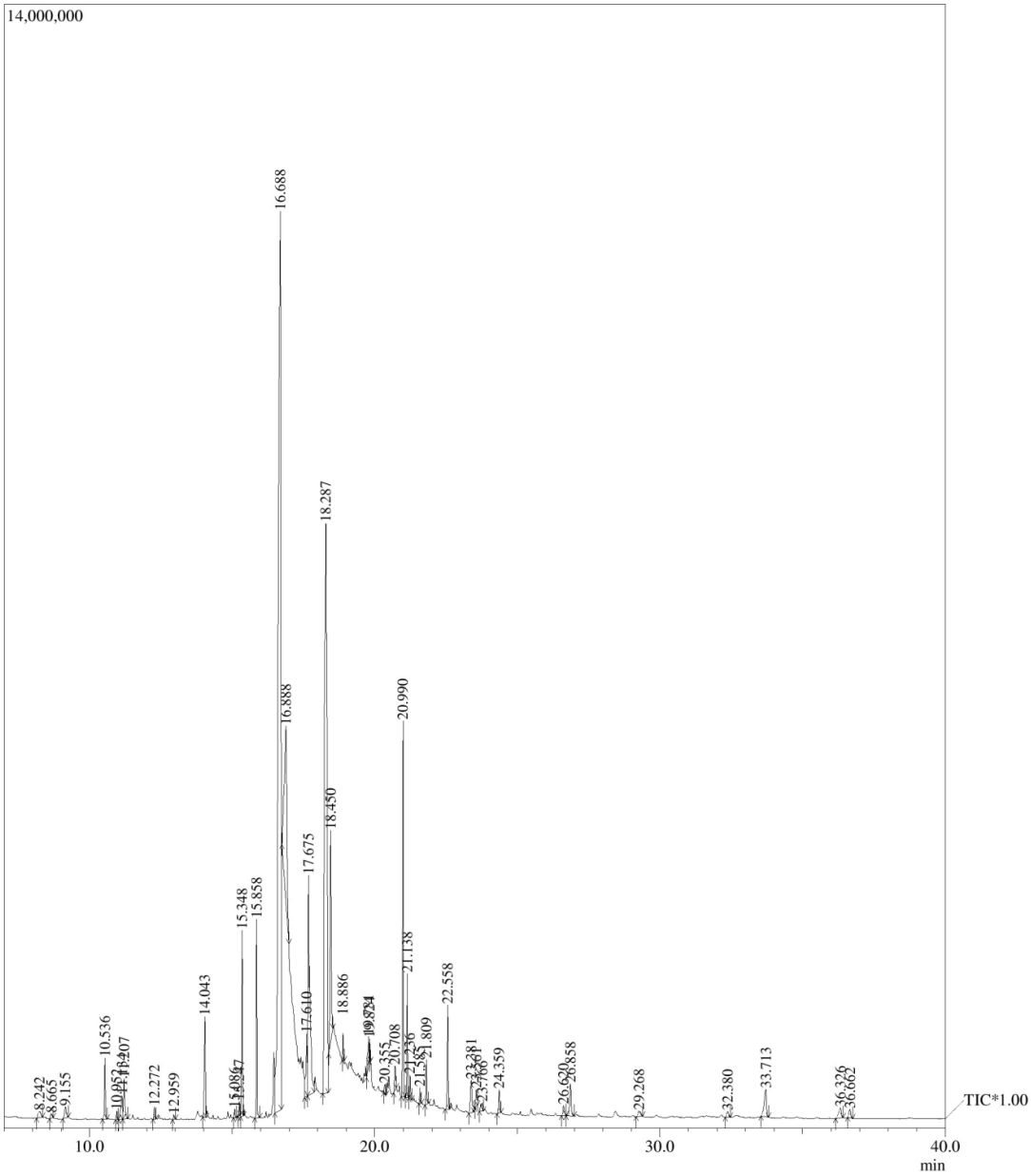

Figure 1
Chromatogram of Fraction 1 (Retention time 7-40 min)

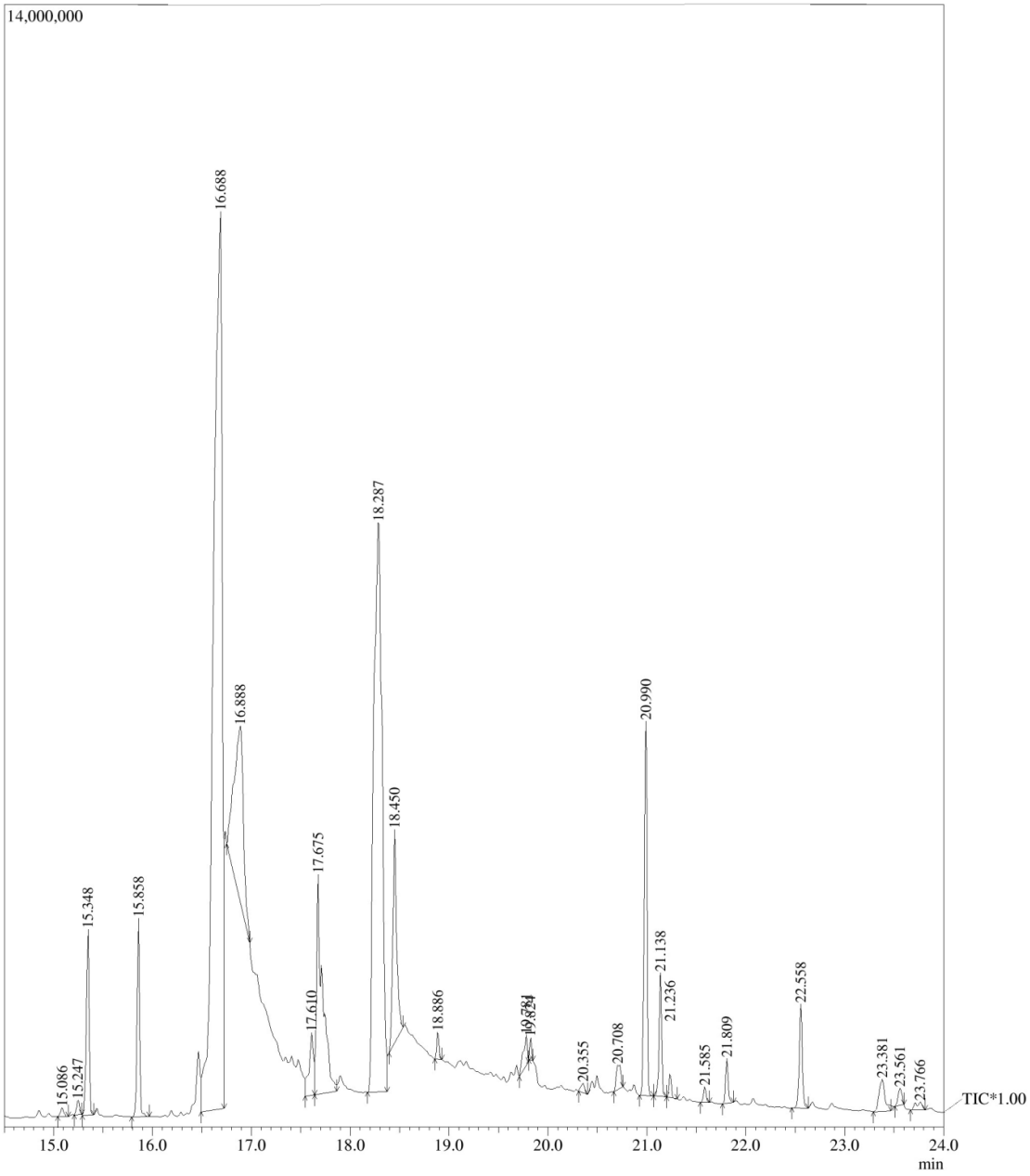

Figure 2
Chromatogram of Fraction 1 (Retention time 14-24 min)

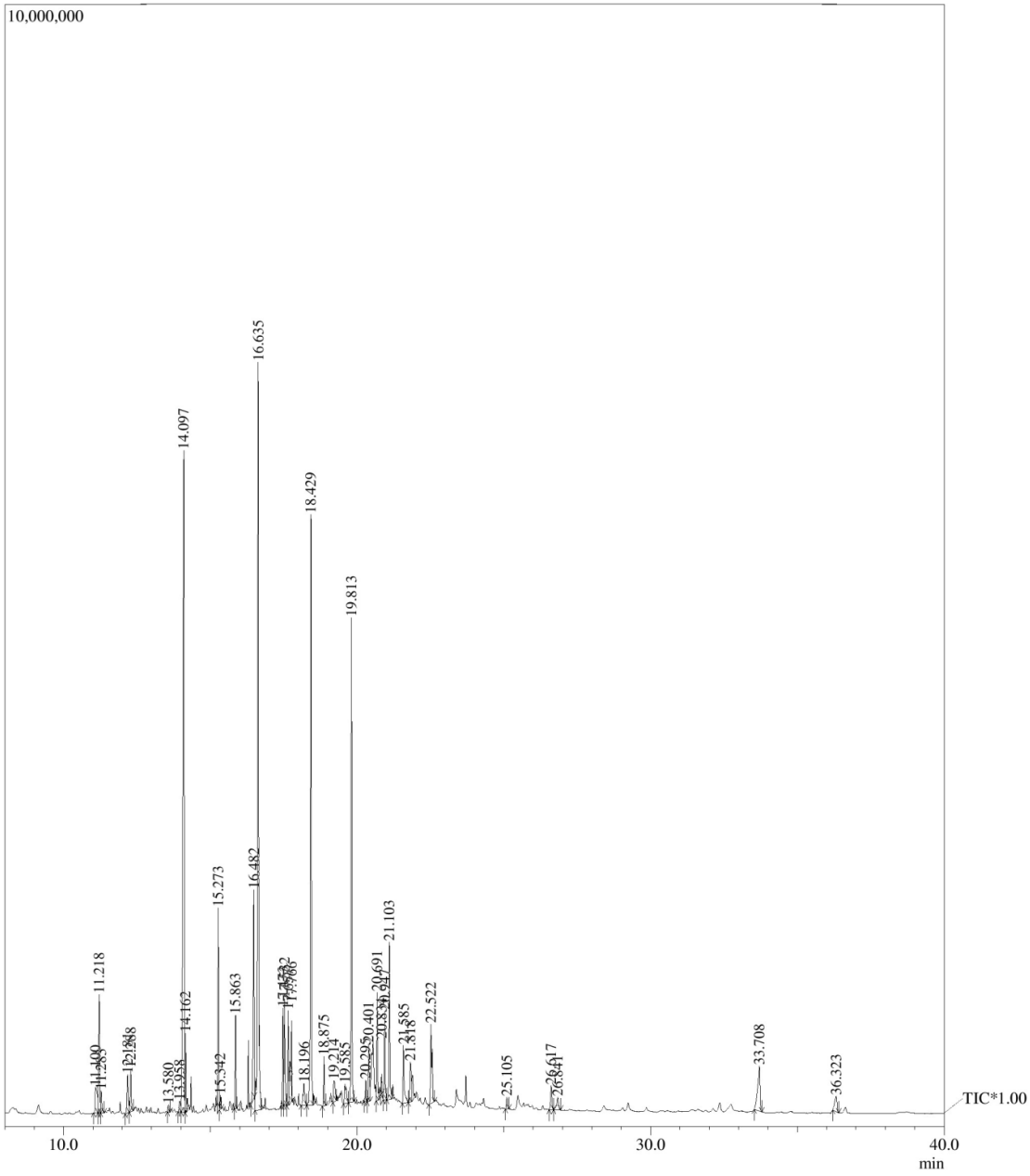

Figure 3
Chromatogram of Fraction 2 (Retention time 7-40 min)

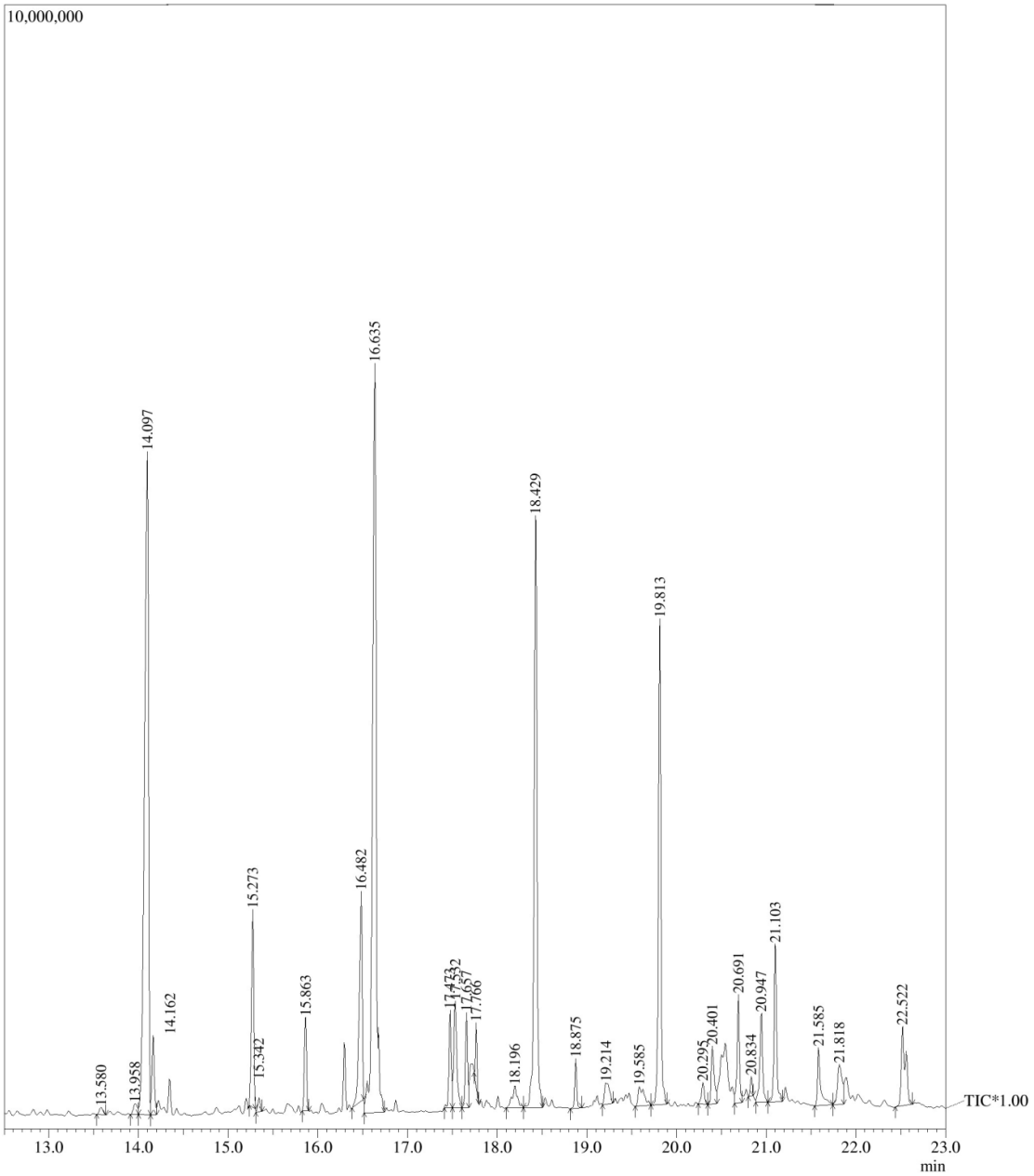

Figure 4
Chromatogram of Fraction 2 (Retention time 13-23 min)

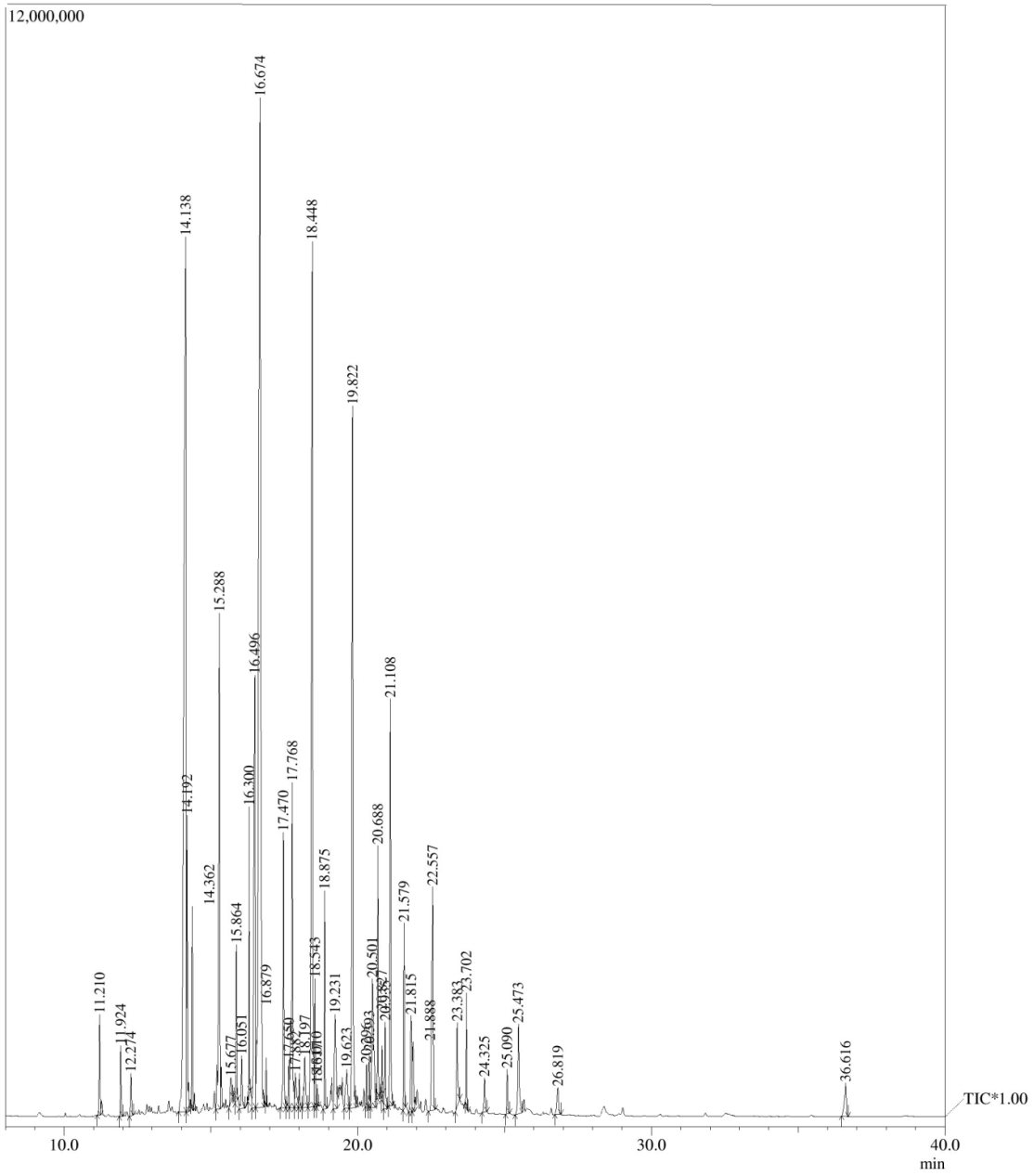

Figure 5
Chromatogram of Fraction 3

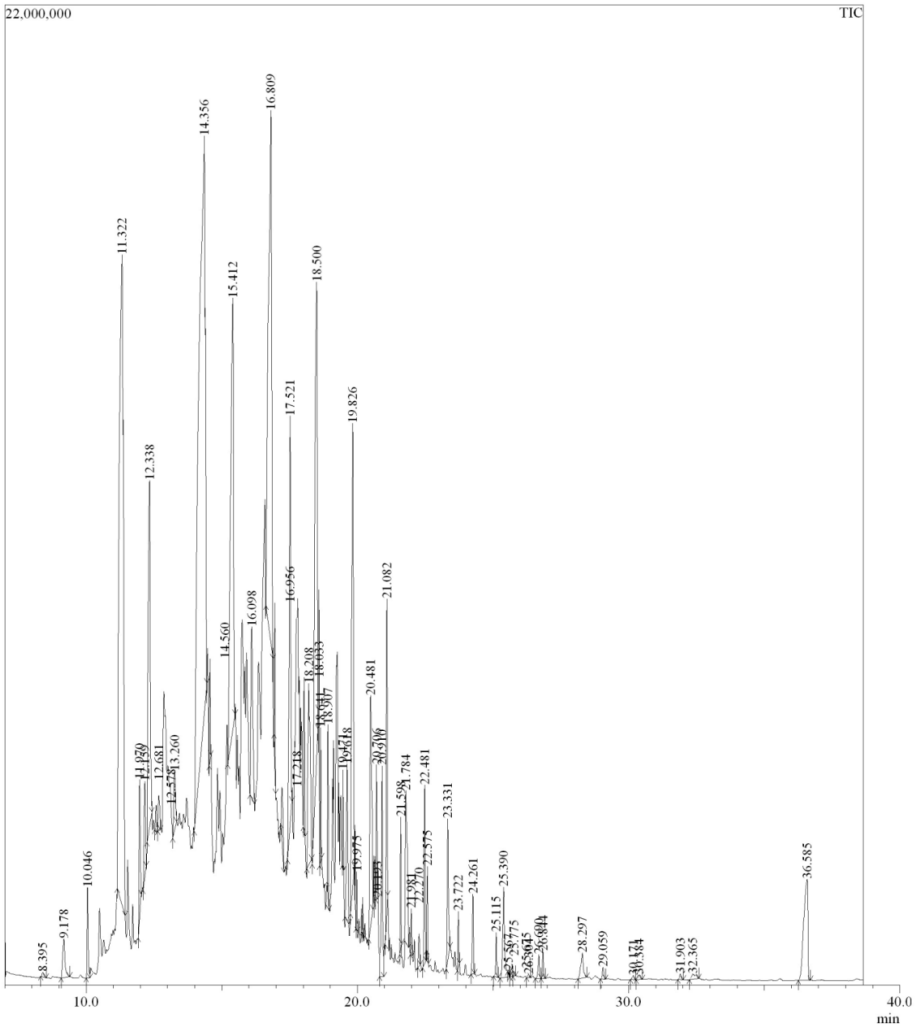

Figure 6
Chromatogram of Fraction 4

Identification of compounds

The identification of compounds present in four fractions of the pet. ether extract was based on direct comparison of the retention time and mass spectral data with those for standard compounds, and by matching with the Wiley and NIST libraries, as well as by comparison of the fragmentation of the mass spectra with those reported in the literature.

RESULTS AND DISCUSSION

Microwave assisted extraction

In this study, MAE of root bark of *Guazuma tomentosa* was carried out. During microwave processing, the moisture inside the plant cell evaporates and generates tremendous pressure on the cell wall due to swelling of the plant cell. The pressure pushes the cell wall from inside, stretching and ultimately rupturing it, which facilitates leaching out of the active constituents from the ruptured cells to the surrounding solvent thus improving the yield of phytoconstituents [17]. The extraction conditions such as microwave power, irradiation tome, solvent composition and loading ratio were optimised to obtain the maximum yield of the extract.

Effect of microwave power

Figure7(a) indicates that during short irradiation time (2 and 5 min), the yield of extract increases with increase in power. But

when this duration is long enough (9 min), the extract yields for different powers are almost same. The maximum yield is obtained at 300 W power, hence it was considered as optimum.

Effect of irradiation time

It is clearly evident from Figure7(b) that there is a rise in extract yield between 2-9 min, but afterwards there was no significant difference in the yields, so the extraction time of 9 min was considered as optimum.

Effect of solvent composition

Figure 7 (c) shows that the concentration of aqueous ethanol greatly influences the extract yield, the highest obtained with 80% v/v ethanol concentration.

Effect of solvent to material ratio

Figure 7 (d) reveals that the extract yield increases up to solvent to material ratio 30 : 1 and thereafter decreases. Thus, it was considered to be optimum.

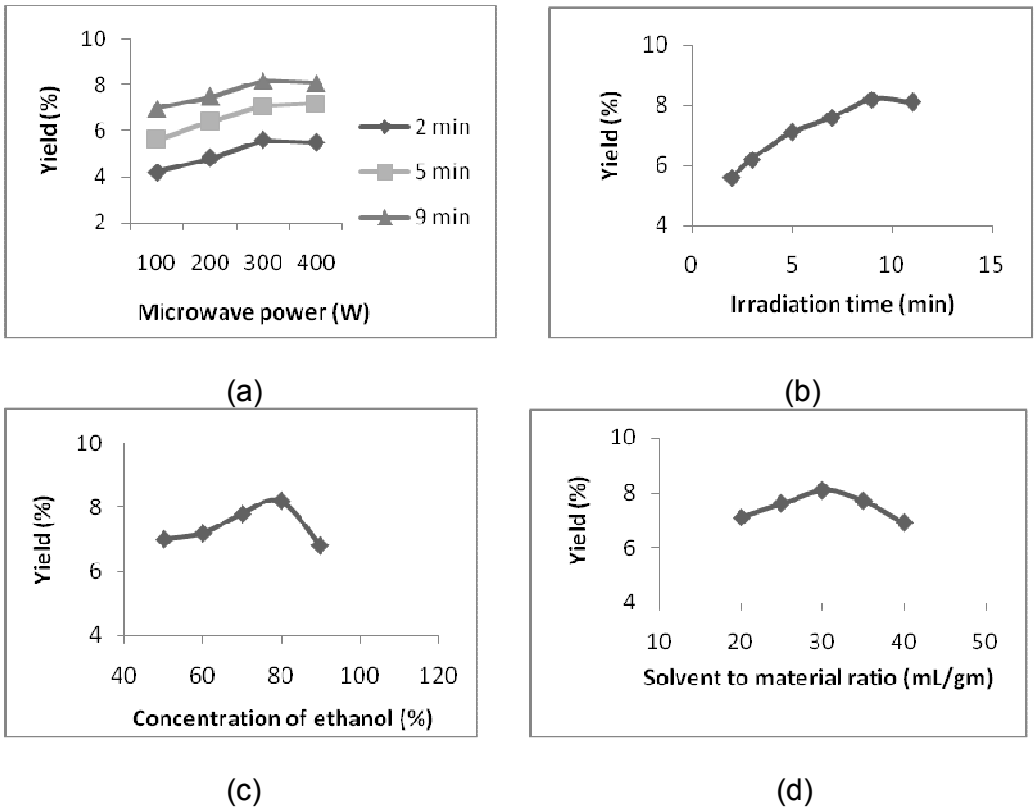

Figure 7

Optimisation of extraction conditions: (a) Effect of microwave power on extract yield, Extraction conditions: 30 mL ethanol as extraction solvent and 5 min preleaching time; (b) Effect of irradiation time on extract yield, Extraction conditions: Microwave power: 300W, 30 mL ethanol as extraction solvent and 5 min preleaching time; (c) Effect of ethanol concentration on extract yield, Extraction conditions: Microwave power: 300W, extraction time: 9 min, 30 mL ethanol as extraction solvent and 5 min preleaching time; (d) Effect of solvent to material ratio on extract yield; Extraction conditions: Microwave power: 300W, extraction time: 9 min, 30 mL ethanol as extraction solvent (concentration: 80% v/v) and 5 min preleaching time.

GC-MS analysis

The pet. ether solubles obtained from the extract on column chromatography over silica gel afforded four fractions which were analysed by GC-MS. The results of the GC-MS analysis led to the identification of a number of long chain compounds, 17 flavonoids, 11 terpenoids, 3 steroids and a

coumarin which are summarised in Tables 1-4. Flavonoids and coumarins are known to possess good antioxidant activity [18] and their presence in the root bark of *G. tomentosa* indicates its utility. Further, to check the reproducibility, we performed the GC-MS analysis of fraction 4 thrice and found that the results obtained are consistent.

Table 1
Components of Fraction 1

S.No.	R.Time	Area %	Molecular Mass	Name of the Component
1	8.242	0.27	158	Nonanoic acid
2	8.665	0.11	152	Camphor
3	9.155	0.32	172	Decanoic acid
4	10.536	0.91	206	3,5-Ditert.butyl phenol
5	10.952	0.10	208	1,2,3-Trimethoxy-5-(2-propenyl) benzene
6	11.134	0.64	200	Dodecanoic acid
7	11.207	0.77	240	1-Hexadecanol
8	11.514	0.06	204	Pethybrene
9	12.272	0.18	158	2,2-Dimethyl-1-octanol
10	12.959	0.06	284	Methyl heptadecanoate
11	14.043	1.61	256	1-Heptadecanol
12	15.086	0.12	156	Citronellol
13	15.247	0.19	152	Geranial
14	15.348	2.29	278	Diisobutyl phthalate
15	15.858	2.18	270	Methyl hexadecanoate
16	16.688	35.22	284	Ethyl hexadecanoate
17	16.888	7.10	652	Ascorbic acid-2,6-dihexadecanoate
18	17.610	1.30	298	Ethyl heptadecanoate
19	17.675	5.81	354	Chlorogenic acid
20	18.287	21.13	428	Trans-p-ferulylalcohol-4-O-(6-(2-methyl-3-hydroxypropionyl) glucopyranoside
21	18.450	3.74	355	Ethyl eicosanoate
22	18.886	0.26	-	Unidentified
23	19.781	0.57	282	6-Tridecyl tetrahydro-2H-pyran-2-one
24	19.824	0.18	504	6,6-Ditetradecyl-6,7-dihydrooxepin-2(3)-H-one
25	20.355	0.15	302	1-Hydroxy-2,3,5-trimethoxy xanthone
26	20.708	0.50	504	Luteolin-acetyl-glucuronide
27	20.990	4.72	390	Bis(2-ethyl hexyl) phthalate
28	21.138	1.43	368	Ethyl docosanoate
29	21.236	0.25	312	n-Octadecyl ethanoate
30	21.585	0.18	456	2β-Hydroxy-15-phenyl-(22,24,26-trimethoxy)-ent-labda-8(17),13(E)-diene
31	21.809	0.52	382	Dihydrodiligustilide
32	22.558	1.36	503	Pelargonidin-3-malonyl-rhamnoside
33	23.381	0.86	418	Cyanidin-3-O-arabinoside
34	23.561	0.28	-	Unidentified
35	23.766	0.22	212	Tetradecanal
36	24.359	0.37	424	β-Amyrone
37	26.620	0.23	430	Cerevisterol
38	26.858	0.77	452	3β-Acetoxy-12-oleanene
39	29.268	0.21	384	Cholest-4-en-3-one
40	32.380	0.15	426	β-Amyrin
41	33.713	0.97	412	4-Stigmast-3-enone
42	36.326	0.40	204	β-Curcumene
43	36.662	0.22	662	Epimedoside A

Table 2
Components of Fraction 2

S.No	R.Time	Area%	Molecular Mass	Name of the Component
1	11.100	1.12	168	4-Hydroxy-3-methoxyphenyl ethanol
2	11.218	2.26	224	1-Hexadecene
3	11.283	0.45	198	1-Tetradecene
4	12.181	0.95	210	6,7,8-Trimethoxy coumarin
5	12.288	0.98	226	8-Pentadecanone
6	13.580	0.17	328	Salvigenin
7	13.958	0.29	196	2-Ethyl-1-dodecene
8	14.097	16.89	266	1-Nonadecene
9	14.162	1.29	254	Octadecane
10	15.273	2.84	270	Norwogonine
11	15.342	0.20	278	Diisobutyl phthalate
12	15.863	1.37	270	Methyl hexadecanoate
13	16.482	3.88	278	Dibutyl phthalate
14	16.635	17.93	322	9-Tricosene
15	17.473	1.46	282	10-Nonadecanone
16	17.532	2.17	270	Echinatin
17	17.657	1.40	294	Methy-9,12-octadecadienoate
18	17.766	0.62	578	Kaempferol-3,3-di-O-rhamnoside
19	18.196	0.74	298	5-Hydroxy-7,8-dimethoxyflavone
20	18.429	10.97	355	Chlorogenic acid
21	18.875	0.60	428	Trans-p-ferulylalcohol-4-0-(6-(2-methyl-3-hydroxypropionyl) glucopyranoside
22	19.214	0.69	396	1-Heptacosanol
23	19.585	0.75	298	1-Eicosanol
24	19.813	7.75	535	Cyanidin-3-malonyl rhamnoside
25	20.295	0.38	302	1-Hydroxy-2,3,5-trimethoxy xanthone
26	20.401	1.16	-	Unidentified
27	20.691	1.60	578	Procyanidin dimer
28	20.834	0.25	302	2,4-Bis(1-phenyl ethyl) phenol
29	20.947	1.61	390	Dioctyl phthalate
30	21.103	2.69	438	1-Triacontanol
31	21.585	1.25	456	2β-Hydroxy-15-phenyl-(22,24,26-trimethoxy)-ent-labda-8(17),13(E)-diene
32	21.818	1.28	242	2-Hexyl-1-decanol
33	22.522	2.27	402	1-(3,4,5-trimethoxyphenyl)-2-O-(4-allyl-2,6-dimethoxyphenyl)-2-propanol
34	25.105	0.25	456	3β-Acetoxy-28-norolean-17-ene
35	26.617	0.61	430	Cerevisterol
36	26.841	0.49	344	3',4',7-Trimethyl quercetin
37	33.708	2.03	412	4-Stigmast-3-enone
38	36.323	0.71	204	β-Curcumene

Table 3
Components of Fraction 3

S. No.	R.Time	Area%	Molecular Mass	Name of the Component
1	11.210	0.79	224	1-Hexadecene
2	11.924	0.41	-	Unidentified
3	12.274	0.35	226	8-Pentadecanone
4	14.138	16.46	252	1-Octadecene
5	14.192	1.98	254	Octadecane
6	14.362	1.22	-	Unidentified
7	15.288	4.80	270	Norwogonine
8	15.677	0.35	308	1-Docosene
9	15.864	1.04	270	Methyl hexadecanoate
10	16.051	0.51	214	2-Hexyl-1-octanol
11	16.300	1.74	550	Liquiritigenin-4'-apiosyl (1-2)glucoside
12	16.496	5.00	278	Diisobutyl phthalate
13	16.674	18.39	266	1-Nonadecene
14	16.879	0.25	340	1-Tricosanol
15	17.470	1.98	282	10-Nonadecanone
16	17.650	0.40	294	Methy-9,12-octadecadienoate
17	17.768	3.24	578	Kaempferol-3,3-di-O-rhamnoside
18	17.882	0.43	396	Methyl pentacosanoate
19	18.010	0.24	156	Citronellol
20	18.197	0.68	369	Methyl ester of Chlorogenic acid
21	18.448	10.78	355	Chlorogenic acid
22	18.543	0.70	312	n-Octadecyl ethanoate
23	18.617	0.13	298	1-Eicosanol
24	18.875	1.15	647	Hexacosanyl oleate
25	19.231	1.29	396	1-Heptacosanol
26	19.623	0.61	214	2-Ethyl-1-dodecanol
27	19.822	6.88	594	Quercetin-3-O-glu-7-O-rhamnoside
28	20.296	0.28	302	1-Hydroxy-2,3,5-trimethoxy xanthone
29	20.393	0.34	-	Unidentified
30	20.501	1.97	452	1-Hentriacontanol
31	20.688	1.50	578	Procyanidin dimer
32	20.827	0.28	302	2,4-Bis(1-phenyl ethyl) phenol
33	20.935	0.75	340	Sinapoyl malate
34	21.108	3.18	438	1-Triacontanol
35	21.579	1.10	596	Eriodictoyl-7-O-(6"-trans-p-coumaroyl)-β-D-glucopyranoside
36	21.815	1.18	242	2-Hexyl-1-decanol
37	21.888	0.67	382	Dihydrodiligustilide
38	22.557	2.61		Unidentified
39	23.383	0.88	450	Dotriacontane
40	23.702	0.72	578	Pelargonidin-3-malonyl rhamnoside
41	24.325	0.35	702	11,20-Didecyl triacontane
42	25.090	0.34	502	Nemorosone
43	25.473	1.12	406	Xanthohumol E
45	36.616	0.56	662	Epimedoside A

Table 4
Components of Fraction 4

S. No.	R.Time	Area%	Molecular Mass	Name of the Component
1	9.172	0.17	172	Decanoic acid
2	10.044	0.22	-	Unidentified
3	11.307	14.16	224	1-Hexadecene
4	11.949	0.73	-	Unidentified
5	12.178	0.33	224	1-Cyclohexyl decane
6	12.336	3.95	226	8-Pentadecanone
7	12.867	0.90	326	Docosanol
8	14.267	26.92	252	1-Octadecene
9	14.510	0.30	266	1-Nonadecene
10	14.908	0.16	270	Norwogonine
11	15.363	8.36	269	Baicalein
12	15.730	1.35	322	9-Tricosene
13	15.824	0.16	280	3-Eicosene
14	15.891	0.26	270	Methyl hexadecanoate
15	16.081	0.79	214	2-Hexyl-1-octanol
16	16.317	1.01	550	Liquiritigenin-4'-apiosyl (1-2)glucoside
17	16.553	4.37	278	Diisobutyl phthalate
18	16.738	12.03	298	1-Eicosanol
19	16.916	0.37	340	1-Tricosanol
20	17.498	2.41	282	10-Nonadecanone
21	17.779	1.69	578	Kaempferol-3,3-di-O-rhamnoside
22	18.211	0.63	369	Methyl ester of Chlorogenic acid
23	18.472	7.14	355	Chlorogenic acid
24	18.560	0.50	312	n-Octadecyl ethanoate
25	18.882	0.47	578	Procyanidin dimer
26	19.242	1.01	396	1-Heptacosanol
27	19.636	0.27	214	2-Ethyl-1-dodecanol
28	19.833	3.78	594	Quercetin-3-O-glu-7-O-rhamnoside
29	20.509	0.98	336	Unidentified
30	20.686	0.56	596	Eriodictoyl-7-O-(6"-trans-p-coumaroyl)-β-D-glucopyranoside
31	20.938	0.33	340	Sinapoyl malate
32	21.106	1.23	438	1-Triacontanol
33	21.577	0.28	578	Kaempferol diglucoside
34	21.818	0.67	242	2-Hexyl-1-decanol
35	22.529	0.63	578	Pelargonidin-3-malonyl rhamnoside
36	23.378	0.31	702	11,20-Didecyl triacontane
37	25.456	0.17	154	1,8-Cineole
38	36.624	0.43	662	Epimedoside A

CONCLUSION

In this study, an effective time saving extraction model using microwaves for the extraction of root bark of *Guazuma tomentosa* followed by the GC-MS analysis of the four fractions of the pet ether extract is presented. The results have shown that the root bark of *Guazuma tomentosa* is a rich source of terpenoids, flavonoids, coumarins and long chain compounds and are thus helpful for the utilization of root bark of *Guazuma tomentosa.*

ACKNOWLEDGEMENTS

The authors are grateful to the AIRF, JNU, New Delhi for the GC-MS facility and to CSIR, New Delhi for financial assistance.

REFERENCES

1. K.R. Kirtikar, and B.D. Basu, Indian Medicinal Plants, Lalit Mohan Basu and Co, Allahabad, (1975).
2. R.N. Chopra, S.L. Nayar, and I.C. Chopra, Glossary of Indian Medicinal Plants CSIR, New Delhi, (1971).
3. Alarcon-Aguilara F.J., Roman-Ramos R., Perez-Gutierrez S., Aguilar-Contreras A., Contreras-Weber C.C and Flores-Saenz J.L., Study of the anti-hyperglycemic effect of plants used as antidiabetics, J Ethnopharmacol, 61: 101-110 (1998).
4. Caballero-George C., Vanderheyden P.M., De Bruyne T., Shahat A.A., Van den Heuvel H., Solis P.N., Gupta M.P., Claeys M., Pieters L., Vauquelin G and Vlietinck A.J., *In vitro* inhibition of [3H]-angiotensin II binding on the human AT1 receptor by proantho cyanidins from *Guazuma ulmifolia* bark, Planta Medica, 68: 1066-1071 (2002).
5. Hör M., Rimpler H and Heinrich M., Inhibition of intestinal chloride secretion by proanthocyanidins from *Guazuma ulmifolia,* Planta Medica, 61: 208-212 (1995).
6. Navarro M.C., Montilla M.P., Cabo M.M., Galisteo M., Cáceres A., Morales C and Berger I., Antibacterial, antiprotozoal and antioxidant activity of five plants used in Izabal for infectious diseases, Phytother Res, 17: 325-329 (2003).
7. Felipe A.M., Rincão V.P., Benati F.J., Linhares R.E., Galina K.J., de Toledo C.E., Lopes G.C., de Mello J.C.P and Nozawa C., Antiviral effect of *Guazuma ulmifolia* and *Stryphnodendron adstringens* on poliovirus and bovine herpesvirus, Biol Pharm Bull, 29: 1092-1095 (2006).
8. Lopes G.C., Bruschi M.L and de Mello J.C.P., RP-LC-UV Determination of Proanthocyanidins in *Guazuma ulmifolia,* Chromatographia, 69: 175-181 (2009).
9. Agarwal O.P., Saxena U., Jain S.C and Jain R., A New Biscoumarin and other constituents from *Guazuma tomentosa* roots, Chem Nat Compds, 46: 713-715 (2010).
10. Anjaneyulu A.S.R and Murthy V.S., Chemical examination of bark of *Guazuma tomentosa* Kunth, Planta Medica, 32: 247-248 (1977).
11. Anjaneyulu A.S.R and Murthy V.S., Chemical examination of the leaves of *Guazuma tomentosa* Kunth, Curr Sci, 46: 776-777 (1977).
12. Seigler D.S., Pauli G.F., Fr□hlich R., Wegelius E., Nahrstedt A., Glander K.E and binger J.E., Cyanogenic glycosides and menisdaurin from *Guazuma ulmifolia*, *Ostrya virginiana*, *Tiquilia plicata* and *Tiquilia canescens,* Phytochemistry, 66: 1567-1580 (2005).
13. Anjaneyulu A.S.R and Murthy V.S., Chemical examination of heartwood of *Guazuma tomentosa* Kunth, Indian J Chem, 20B: 85-87 (1981).
14. Subramanian S.S and Swamy M.N., Flavonoids of the flowers of *Guazuma tomentosa* and *Delonix elata,* Curr Sci, 32: 308-310 (1963).
15. Desai M., Parikh J and Parikh P.A., Extraction of natural products using microwaves as a heat source, Separation Purification Rev, 39: 1-32 (2010).
16. Zhang H.F., Yang X.H and Wang Y., Microwave assisted extraction of secondary metabolites from plants: Current status and future directions, Trends in Food Sci Technol, 22: 672-688 (2011).
17. Mandal V., Dewanjee S and Mandal S.C., Microwave-assisted extraction of total bioactive saponin fraction from *Gymnema sylvestre* with reference to gymnemagenin: a potential biomarker, Phytochem Anal, 20: 491-497 (2009).
18. Yu J., Wang L., Walzem R.L., Miller E.G., Pike L.M and Patil B.S., Antioxidant activity of citrus limonoids, flavonoids and coumarins, J. Agric. Food Chem., 53: 2009-2014 (2005).