

Original Research Article

Analysis of Bioactive Compounds in Latex of *Croton bonplandianum* (Baill.) using GC-MS

ABSTRACT

Background: *Croton bonplandianum* Baill. belongs to the family of Euphorbiaceae and commonly known as 'Bantulsi'. Traditionally, the latex of this plant is used for cuts and wounds.

Objective: The present investigation was designed to determine the bioactive constituents of latex of *C. bonplandianum* by GC-MS. In this study, the mass spectrum of the compounds found in the ethanolic extract of latex was matched with the National Institute of Standards and Technology (NIST) library.

Place and Duration of Study: Post Graduate and Research Department of Biochemistry at Government Arts College (Autonomous), Kumbakonam and Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Tirumalaisamudram, Thanjavur, Tamilnadu, India, during the months between October to December, 2015.

Methodology: 10 ml of fresh latex was mixed with 10 ml of ethanol and kept at shaker for 3 hours. The sample was filtered and concentrated through nitrogen flushing to 1 ml. From this, 2 µl of prepared sample was injected into GC-MS instrument for phytochemical analysis.

Results: The GC-MS analysis of the ethanolic extract of latex of *C. bonplandianum* revealed that the presence of twenty three phytochemicals including the anti-inflammatory, antioxidant and antimicrobial compounds but not all of them are bioactive compounds. The major chemical constituents present in the latex are 2-C-methyl-myoinositol (32.4), mequinol (0.74), 4-methylphenol (6.86), 1,2,3-benzenetriol (3.54), 3-methyl-quinoline, (0.44), n-hexadecanoic acid [palmitic acid] (5.36) and octadecanoic acid [stearic acid] (1.95).

Conclusion: Further study is needed to isolate, identify, characterize and elucidate the structure of bioactive compounds responsible for therapeutic values of latex of *C. bonplandianum*.

Keywords: *Croton bonplandianum*, latex, GC-MS, ethanolic extract, Bantulsi

1. INTRODUCTION

Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential. The search on medicinal plants has led to the discovery of novel drug candidates used against diverse diseases [1]. According to the World Health Organization [2], more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Phytochemicals are natural bioactive compounds found in plants such as vegetables, fruits, medicinal plants, flowers, leaves and roots that act among these as a defense system against diseases [3]. Screening of biologically active medicinal compounds has been conducted on well-known species of plants used in traditional medicines [4]. In the recent years GC-MS has been applied to unambiguously identify the structures of different phytoconstituents in plant extracts and biological samples with great success [5,6]. GC-MS is one of the best techniques to identify the constituents of volatile matter such as long and branched chain hydrocarbons, alcohols, acids, esters etc [7]. The phytochemical screening by GC-MS analysis proved that the plants are pharmaceutically important due to presence of different medicinally important phytochemical compounds and secondary plant metabolites. The GC-MS analysis of the plants showed that the presence of important bioactive compounds.

The plant latex is a natural polymer composed of 320 to 35,000 isoprene molecules and it is stored in specialized cells called laticifer [8,9]. The latex is known to be phytochemically diverse that contains complex mixtures of terpenoids, phenolics, proteins and alkaloids [10]. Although, all parts of the plant

phytochemical
↑

phytochemicals

remove
lupulin
and close
up.

Matrix
lower case

have been used for medicinal purposes [11,12]. Comprehensive literature survey on the plant latex revealed that it contains wide diversities of defense chemicals and proteins, which displayed different biological activities [13,14]. Moreover, some compounds with wound healing properties were reportedly found in the plant latex [11]. *C. bonplandianum* belongs to the family of Euphorbiaceae. It is widely distributed throughout the plains of India. It is a small perennial herb, green and laticiferous growing upto 1-2 ft tall [15]. *C. bonplandianum* is also well known for its medicinal value. Seeds of *C. bonplandianum* are used for the treatment of jaundice, acute constipation, abdominal dropsy and internal abscesses [16]. Its ethanolic extract has been found to possess hypotensive, spasmolytic and antimicrobial activities [17,18]. The latex of *C. bonplandianum* is used to treat cuts and wounds traditionally [19,20]. The latex of *C. bonplandianum* (1:5 v/v in 50% acetone) showed antifungal activity [21]. Many pharmacological studies are available using latex of *C. bonplandianum*. But there is no study on the screening of bioactive compounds of latex of *C. bonplandianum*. Therefore, the present study was aimed to investigate the phytoconstituents of ethanolic extract of latex of *C. bonplandianum* by GC-MS.

2. MATERIALS AND METHODS

2.1. Collection of Plant Material

C. bonplandianum latex was collected from the waste lands in and around Mayiladuthurai, Nagapattinam District, Tamilnadu, India, where it was found naturally. The plant was identified by Rev. Dr. S. John Britto, Director, Rabinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamilnadu, India. The latex of *C. bonplandianum* was obtained as exudates by and plucking of fresh leaves from actively growing plants. The latex was collected into sterile, plastic containers by pressing and squeezing in between fingers, the apex of the leaves to release as much as possible latex into the containers. The collections of latex were made in the mornings [22]. After collection, the latex was centrifuged with 5000 rpm for 15 minutes to remove any solid particles present in it. The latex was decanted off into containers and plugged with cotton and stored at 4°C until the time of use.

2.2. Identification of Phytocompounds by GC-MS

2.2.1. Sample Preparation

10 ml of fresh latex was mixed with 10 ml of ethanol and kept in the orbital shaker for overnight [23]. The sample was filtered and concentrated through nitrogen flushing to 1 ml. From this, 2 µl of sample was injected into GC-MS instrument for phytochemical analysis.

2.2.2. Equipment

GC-MS analysis was carried out by using a Perkin Elmer Clarus 500 for the screening of phytocompounds of *C. bonplandianum* [24]. The data were obtained on Capillary Column Elite- 5MS (5% phenyl 95% dimethyl poly siloxane). Helium was used as the carrier gas with a flow rate of 1ml/min in the split mode (10:1). An aliquot of 2 µl of ethanol solution of the sample was injected into the column with the injector temperature at 250°C. GC oven temperature started at 110°C and holding for 2 min and it was raised to 200°C at the rate of 10°C/min without holding. Holding was allowed at 280°C for 9 min with program rate of 5°C/min. The injector and detector temperatures were set at 250°C and 280°C, respectively. GC interface and ion source temperature was maintained at 200°C. The mass spectrum of compounds in the sample was obtained by electron ionization at 70 eV and the detector was operated in scan mode from 40-450 amu (atomic mass units). A scan interval of 0.5 second and fragments from 40 to 450 Da were maintained. The total running time was 36 minutes.

2.2.3. Interpretation of Mass Spectrum (MS)

In the MS Programme, NIST Version 2.0 library database of National Institute of Standard and Technology (NIST) having more than 2, 00,000 patterns were used for identifying the phytocomponents of *C. bonplandianum*. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the *C. bonplandianum* were ascertained.

3. RESULTS AND DISCUSSION

Nowadays the search on the phytochemicals of medicinal plants with their activity has increased. The combination of the best separation technique (GC) with the best identification technique (MS) made GC-MS and it is an ideal technique for screening of volatile and semi-volatile bioactive compounds [25]. In the present study, twenty three bioactive chemical compounds in the latex of *C. bonplandianum* were identified and their names, retention time (RT), peak area percentage and molecular weight are represented in Table 1. The highest peak area percentage was obtained by 2-C-methylmyoinositol (32.4%) with the retention time of 10.48 and the lowest peak area percentage was obtained by N-ethyl-4-methoxy-1,3,5-Triazin-2-amine (0.07%) with the retention time of 23.31. The most abundant components were found in the latex are 2-C-methylmyoinositol (42.40%), 13-octadecenal (23.31%), 2-methoxy-4-methylbenzoic acid (10.90%), D-allose (10.63%) and 4-methylphenol (6.86%).

In our previous study, 10 ml of latex was mixed with 90 ml of ethanol and prepared the extract as mentioned in the section 2.2.1 and then carried out GC-MS analysis. In that study, the latex extract of *C. bonplandianum* showed the presence of only one compound 2-C-methylmyoinositol (30.8%) [26]. But in the present study, the extract of latex of *C. bonplandianum* showed that the presence of twenty three compounds including 2-C-methylmyoinositol (32.4%). This variation may be due to the dilution of the latex with ethanol because in this study the latex was mixed with equal volume of ethanol, but in the previous study the latex was mixed with 9 fold volume of ethanol for extract preparation. So, the present study confirmed that the dilution of the latex play a main role in the presence of phytochemicals and appearance of peaks in the GC-MS chromatogram.

The biological activities of identified phytochemicals in the latex extract of *C. bonplandianum* were predicted based on Dr. Duke's phytochemical and ethnobotanical databases [27]. Among the identified phytochemicals in the latex of *C. bonplandianum*, a few compounds like 2-C-methylmyoinositol (32.4%), 3-methylquinoline (0.44%), 4-amino-6-methylpiperidin-2-one (5.24%), 1,2,3-benzenetriol (3.54%), 2-methoxy-4-methylbenzoic acid (10.90%) and n-hexadecanoic acid (5.36%) possess antimicrobial activity. The phytochemical 1,2,3-benzenetriol (pyrogallol) is an important allelochemical belongs to phenolic compound present in the plants and it is used in controlling bacterial diseases [28].

The phytochemicals Mequinol (0.74%), 4-methylphenol (6.86%) and 3-methylquinoline (0.44%) have anti-inflammatory activity. Mequinol is a common active ingredient in topical drugs used for skin depigmentation and it is a derivative of hydroquinone (4-hydroxyanisole, hydroquinone monomethyl ether). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. A number of studies have focused on the phytochemicals of biological properties such as antiapoptosis, anti-ageing, anticarcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of the endothelial functions, as well as inhibition of angiogenesis and cell proliferation [29]. Phenolic compounds have been extensively used in disinfections and as bactericides [30]. Quinoline compounds have excellent antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* [31].

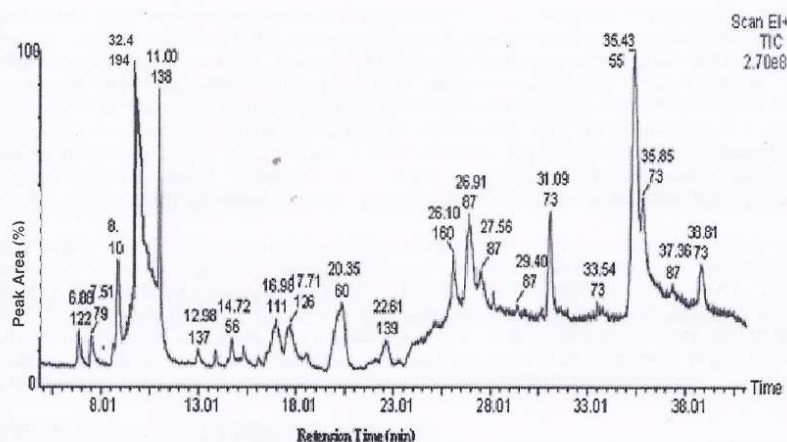
Based on the Dr. Duke's phytochemical and ethnobotanical databases, the 2-methoxy-4-methylphenol (9.55%), D-allose (10.63%), 6-methoxy-1H-indole-3-carbaldehyde (5.98%) and 2-methoxy-4-methylbenzoic acid (10.90%) have antioxidant activity. The n-hexadecanoic acid (5.36%) and 2-methoxy-1-naphthyl thiocyanate (2.58%) have antitumor activity and 3-methylquinoline (0.44%) has antimalarial activity.

The antioxidant properties of phenolic compounds are due their redox properties, ability to chelate metals and quenching of singlet oxygen [32]. Natural phenolic compounds have great potential as natural antimicrobials and food preservatives [33]. D-allose is a novel antitumor monosaccharide, which causes cell growth inhibition on cancer cells by inducing the tumor suppressor gene [34]. The n-hexadecanoic acid has anti-tumor activity against human leukemic cells as well as murine cells [35]. The fatty acids exhibit cytotoxicity against HeLa cells and retard tumor growth [36]. The n-hexadecanoic acid and

139 octadecanoic acid were showed significant cytotoxicity against oral cancer (KB), breast cancer (MCF-7)
140 and lung cancer (NCI-H187) [37].
141

142 The peak area of 1.54% was obtained by benzyl alcohol and it occurs naturally in many plants and it has
143 strong activity against gram positive bacteria and has weak activity against gram negative bacteria,
144 yeasts and moulds [38]. Benzyl alcohol has mild local anesthetic and anti-spasmodic properties [39].
145

146 In addition, the results of the GC-MS profile of latex of *C. bonplandianum* may be used as
147 pharmacognostical tool for the identification of bioactive compounds. The presence of various bioactive
148 compounds may be responsible for the therapeutic value of latex of *C. bonplandianum* to treat various
149 ailments by traditional practitioners. However, further study is needed to isolate the individual
150 phytochemical compound from the latex of *C. bonplandianum* and it may be helpful to find a novel drug.
151



154
155
156 Fig. 1. GC-MS chromatogram of ethanolic extract of latex of *C. bonplandianum*
157

Table 1. Screening of the phytocompounds of ethanolic extract of latex of *C. bonplandianum* by GC-MS // *italics*

Sl. No.	RT	Name of the compound	Molecular Formula	MW	Peak %	Area	Nature of compound	**Activity
1.	6.88	1-Methoxy-1,3,5-cycloheptatriene	C ₈ H ₁₀ O	122	1.52		<i>ether</i> compound	No activity reported // <i>alkene ether</i>
2.	7.51	Benzyl alcohol	C ₇ H ₈ O	108	1.54		Alcoholic compound	Antibacterial
3.	8.62	Mequinol	C ₇ H ₈ O ₂	124	0.74		Phenolic compound	Anti-inflammatory
4.	8.87	4-Methylphenol	C ₇ H ₈ O	108	6.86		Phenolic compound	Anti-inflammatory // <i>remove hyphen and close up</i>
5.	10.48	2-C-Methylmyoinositol	C ₇ H ₁₇ O ₆	194	32.4		Sugar derivative	Antimicrobial // <i>remove hyphen and close up</i>
6.	11.00	2-Methoxy-4-methylphenol	C ₈ H ₁₀ O ₂	138	9.55		Phenolic compound	Antioxidant Cytotoxicity // <i>remove hyphen and close up</i>
7.	12.98	4-Ethyl-2-methoxy-phenol	C ₉ H ₁₂ O ₂	152	0.77		Phenolic compound	No activity reported
8.	13.90	2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150	0.54		Phenolic compound	No activity reported
9.	14.72	4,4-Dimethyl-1,3-cyclopentanedione	C ₇ H ₁₀ O ₂	126	1.01		Ketone compound	Herbicidal activity
10.	15.33	3-Methylquinoline	C ₁₀ H ₉ N	143	0.44		Alkaloid compound	Antimalarial Antibacterial Antifungal Anti-inflammatory // <i>remove hyphen and close up</i>
11.	16.93	4-Amino-6-methyl-piperidin-2-one	C ₈ H ₁₂ N ₂ O	128	5.24		Alkaloid compound	Antimicrobial
12.	17.71	1,2,3-Benzenetriol	C ₆ H ₆ O ₃	126	3.54		Phenolic compound	Antimicrobial
13.	18.55	1H-Indol-5-ol	C ₈ H ₇ NO	133	0.35		<i>alkaloid</i> Phenolic compound	No activity reported // <i>phenolic, alkaloid</i>
14.	20.34	D-Alose	C ₆ H ₁₂ O ₆	180	10.63		Sugar compound	Antiproliferative, Antioxidant Endocytic

4. CONCLUSION

In the present study, the GC-MS analysis of ethanolic extract of latex of *C. bonplandianum* revealed that the presence of twenty three phytochemicals. According to the Dr. Duke's phytochemical and ethnobotanical databases, eight compounds have antimicrobial, three compounds have antiinflammatory, five compounds have antioxidant, two compounds have antitumour and one compound has antimalarial activities. Thus, the GC-MS analysis is the first step towards understanding the nature of active principles in the latex of *C. bonplandianum* and this study may also be helpful to further research related to phytopharmacology.

REFERENCES

1. Vital PG, Rivera WL. Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L. f.) King and Robinson and *Uncaria perrottetii* (A. Rich) Merr extracts. *J Medicinal Plants Res.* 2009; 3(7):511-518.
2. World Health Organization (2008). Traditional medicine (Online). <http://www.int/medicines/areas/traditional/en/index.html>.
3. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine – A move towards nature. *Biotechnol Mol Biol Rev.* 2007; 1(4): 097-104.
4. Sulekha Joshi, Gupta VP, Utkarsh Sharma. Phytochemical Screening of medicinal plant *Dolichandrone falcata*. *Biological Forum – An International Journal.* 2016; 8(1): 215-220.
5. Prasain JK, Wang CC, Barnes S. Mass spectrometric methods for the determination of flavonoids in biological samples. *Free Radic Biol Med.* 2004; 37(9):1324-1350.
6. De Rijke E, Out P, Neissen WMA, Ariese F, Gooijer C, Brinkman UA. The analytical separation and detection methods for flavonoids. *J Chromatogr A.* 2006; 1112(1-2):31-63.
7. Anjali Ruikar, Rasika Torane, Amruta Tambe, Vedavati Puranik, Nirmala Deshpande. GC-MS study of a steam volatile matter from *Mimusops elengi*. *International Journal of Chem Tech Research.* 2009; 1(2):158-161.
8. Kang H, Kang MY, Han KH. Identification of natural rubber and characterization of rubber biosynthetic activity in Fig tree. *Plant Physiol.* 2000; 123(3):1133-1142.
9. Upadhyay RK. Plant latex: A natural source of pharmaceuticals and pesticides. *Int J Green Pharm.* 2011; 5(3):169-180.
10. Agrawal AA, Konno K. Latex: A model for understanding mechanisms, ecology and evolution of plant defense against herbivory. *Annu Rev Ecol Evol Syst.* 2009; 40:311-315.
11. Duke JA. Medicinal plant science. London: Macmillan press limited. 1985.
12. Gubitz GM, Mittelbach M, Trabi M. Exploitation of the tropical oil seed plant *Jatropha curcas* L. *Bioresour Technol.* 1999; 67(1):73-82.
13. Konno K. Plant latex and other exudates as plant defense systems: Roles of various defense chemicals and proteins contained therein. *Phytochem.* 2011; 72(13):1510-1530.
14. Kitajima S, Kamei K, Taketani S, Yamaguchi M, Kawai F, Komatsu A, Inukai Y. Two chitinase-like proteins abundantly accumulated in latex of mulberry show insecticidal activity. *BMC Biochem.* 2010; 11:6-11.
15. Sethi A, Sharma RA. Micropropagation of *Croton bonplandinum* Baill. *Int Res J Pharm.* 2011; 2(10):82-86.
16. Reddy KR. Folk medicines from Chittoor District, Andhra Pradesh, used in the treatment of jaundice. *Int J Crude Drug Res.* 1988; 26:137-140.
17. Mishra D, Chaturvedi RV, Tripathi SC. The fungitoxic effect of the essential oil of the herb *Nardostachys jatamansi* D.C. *Trop Agri.* 1995; 72(1):48-52.
18. Verma SP, Kannaiya RS, Upadhyay RR. Antifungal activity of some Euphorbiaceaeous plants. *Ind J Plant Path.* 1998; 16(1-2):62-63.
19. Chandel KPS, Shukla G, Sharma N. Biodiversity in medicinal and aromatic plants in India, Conservation and utilization, New Delhi, National Bureau of Plant Genetic Resources. 1996: 361.
20. Das AJ, Dutta BK, Sharma GD. Medicinal plants use by different tribes of Cachar District, Assam. *Indian J Tradit Know.* 2008; 7(3):446-454.

- 222 21. Asthana A, Mall HV, Dixit K, Gupta S. Fungitoxic properties of latex of plants with special
223 reference to that of *Croton bonplandianum* Baill. Int J Crude Drug Res. 1989; 27:25-28.
- 224 22. Kareem SO, Akpan I, Osho MB. *Calotropis procera* (Sodom apple) – A potential material for
225 enzyme purification. J Biosci Technol. 2003; 87:133-135.
- 226 23. Varsha Jadhav, Vaibhav Kalase, Poonam Patil. GC-MS analysis of bioactive compounds in
227 methanolic extract of *Holigarna grahamii* (Wight) Kurz. Int J Herb Med. 2014; 2(4):35-39.
- 228 24. Janakiraman N, Johnson M, Sahaya Sathish S. GC-MS analysis of bioactive constituents of
229 *Peristrophe bicalyculata* (Retz.) Nees. (Acanthaceae). Asian Pac J Trop Biomed. 2012; S46-S49.
- 230 25. Cowan MM. Plants products as antimicrobial agents. Clin Microbial Rev. 1999; 12(4): 564- 582.
- 231 26. Vennila V, Udayakumar R. GC-MS Analysis of leaf, fruits and latex of *Croton bonplandianum*
232 Baill. Int J Biochem Res Rev. 2015; 5(3):187-197.
- 233 27. Dr. Duke's Phytochemical and Ethnobotanical Databases, Agricultural Research Service of the
234 US Department of Agriculture. <http://www.ars-grin.gov/duke> 16 June 2014.
- 235 28. Ismail Kocacaliszana, Ismet Talanb, Irfan Terzic. Antimicrobial activity of catechol and pyrogallol
236 as allelochemicals. Naturforsch. 2006; 61c:639-642.
- 237 29. Han X, Shen T, Lou H. Dietary polyphenols and their biological significance. Int J Mol Sci. 2007;
238 8:950-988.
- 239 30. Okwu DE. Evaluation of the chemical composition of indigenous species and flavouring agents.
240 Global J Pure Appl Sci. 2001; 8:455-459.
- 241 31. Pintilie Lucia. Quinolones: Synthesis and Antibacterial Activity. Antimicrobial Agents. 2012; 255-
242 272.
- 243 32. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids
244 and phenolic acids. Free Radic Biol Med. 1996; 20:933-956.
- 245 33. Hayriye Cetin-Karaca. Evaluation of natural antimicrobial phenolic compounds against foodborne
246 pathogens. University of Kentucky Master's Theses. Paper 652.
247 http://uknowledge.uky.edu/gradschool_theses/652.
- 248 34. Yamaguchi F, Kamitori K, Sanada K, Horii M, Dong Y, Sui L, Tokuda M. Rare sugar D-allose
249 enhances anti-tumor effect of 5-fluorouracil on the human hepatocellular carcinoma cell line HuH-
250 7. J Biosci Bioeng. 2008; 106(3):248-52.
- 251 35. Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J. Antitumor activity of palmitic acid
252 found as a selective cytotoxic substance in a marine red alga. Anticancer Res. 2002; 22:2587-
253 2590.
- 254 36. Abdel Aziz A, Rady HM, Amer MA, Kiwan HS. Effect of some honey bee extracts on the
255 proliferation, proteolytic and gelatinolytic activities of the hepatocellular carcinoma Hepg2 cell line.
256 Aust J Basic Appl Sci. 2009; 3:2754-2769.
- 257 37. Keawsa-Ard S, Liawruangrath B, Liawruangrath S, Teerawutgulrag A, Pyne SG. Chemical
258 constituents and antioxidant and biological activities of the essential oil from leaves of *Solanum*
259 *spirale*. Nat Prod Commun. 2012; 7:955-958.
- 260 38. Benzyl alcohol. Committee for veterinary medical products. The European Agency for the
261 Evaluation of Medicinal Products Veterinary Medicines Evaluation Unit. 1997.
- 262 39. Srinivasan GV, Sharanappa P, Leela NK, Sadashivam CT, Vijayan KK. Chemical composition
263 and antimicrobial activity of the essential oil *Leuca indica* Merr. flowers. Nat Prod Radiance. 2009;
264 8(5):488-493.
- 265

* To be consistent in citing references, the authors can either include the volume and the issue number in all publications which may be difficult.

OR
Leave out the issue number in all citation but keep the volume number, which is ~~more~~ easier to do.

It is inconsistent to have some publications with only volume number and yet others with both the volume and issue numbers.