ISOLATION OF THE GALLIC ACID IN THE BUTANOLIC FRACTION OF *Crassula ovata* (Mill.) DRUCE (CRASSULACEAE) LEAVES AND ITS VASO-RELAXING EFFECT

|Nantenaina Tombozara [1] | Zoarilala Rinah Razafindrakoto [2]| David Ramanitrahasimbola [2,3] | Reine Dorothée Ramilison Razafimahefa [1] | Eric Marchioni [4] | and | Dina Andriamahavola Rakotondramanana [3*] |

[1.] University of Antananarivo | Faculty of Sciences, Organic Chemistry Department | Laboratory of Applied Chemistry to Natural Substances | Antananarivo | Madagascar |
[2.] Malagasy Institute of Applied Research | Laboratory of Applied Pharmacognosy | Itaosy | Madagascar |
[3.] University of Antananarivo | Faculty of Medicine, Pharmacy Department | Antananarivo | Madagascar |
[4.] University of Strasbourg | Laboratory of Analytical Chemistry of Bioactive Molecules | Strasbourg | France |

|Received | 23 April 2017| |Accepted | 07 May 2017| |Published 15 May 2017 |

ABSTRACT

Backgroud: *Crassula ovata* (Mill.) is a medicinal plant traditionally used to treat high blood pressure in Madagascar. **Objectives:** This work aims to determine the pharmacological mechanism of its antihypertensive virtue, to identify and isolate the major compound and to elucidate its chemical structure. **Method:** The powder of dried leaves of *C. ovata* was extracted by maceration with 90% ethanol. The ethanol solution was depigmented by activated charcoal then filtered on Whatman's filter paper and evaporated to dryness. This ethanol crude extract was dissolved in distilled water and then successively partitioned with dichloromethane, ethyl acetate and butanol. Two serials of column chromatography on silica gel eluted by an increasing polarity solvent system and some preparative chromatography were used to isolate the major compound of the butanol fraction. The chemical structure of the purified compound was elucidated using some spectrometric methods UPLC–MS, UPLC–UV, 1D and 2D NMR. The vaso-relaxing activity of each extract, fraction and product was assessed on the phenylephrine pre-contacted isolated rat aorta. **Result:** The ethanol crude extract exhibited a vaso-relaxing activity with an EC_{50} 247.04±5µg/ml. Among the obtained four fractions, the aqueous one showed a more potent vaso-relaxing activity but the major compound was found in the butanol fraction. It was identified as a 3,4,5-trihydroxybenzoic acid or a gallic acid. It exhibits an interesting vaso-relaxing activity with an EC_{50} 38.8±1µg/ml. **Conclusion:** The vaso-relaxing effect of the *C. ovata* leaves crude extract certainly contributes on the antihypertensive virtue of this medicinal plant. Gallic acid constitutes the major compound of its leaf. Its vaso-relaxing activity is at about 6 times more potent than that of the crude extract.

***Key words**: Crassula ovata, vaso-relaxing activity, Gallic acid, NMR, Spectrometric methods*

1. INTRODUCTION

According to the statistic published by the Malagasy Ministry of Health, the high blood pressure actually represents one of the major concerns of the public health. Its prevalence is estimated to 28.5% of the Malagasy adult population [1]. In the most case, especially for the patients living in the rural zones, the management of the arterial hypertension is generally by some medicinal plant tisane taken daily, a decoction or an infusion preparation. In the Malagasy folk medicine, the tisane prepared by decoction of *C. ovata* leaves is used to treat high blood pressure and can alleviate headache, dizziness, stomachache and liver injury. The aims of this work are to investigate the pharmacological activity of this plant on the vascular tissue which could explain its traditional therapeutic virtue as an anti-hypertensive plant, to isolate the bioactive compound and to determine its chemical structure.

Crassula ovata, an ornamental plant originated from South Africa [2,3,4,5,6], belongs to CRASSULACEAE family. It is known as a jade plant because of the jade green color of its leaves [4]. Its Malagasy vernacular name is reozo or baobabakely, literally a dwarf baobab because of its vegetal form. *C. ovata* has evergreen leaves, thick, smooth, rounded branches and fleshy leaves that grow in opposite pairs along the branches. Some varieties may develop a red tinge on the edges of leaves when exposed to high levels of solar radiation.

2. MATERIALS AND METHODS

2.1 Plant material

Leaves of *C. ovata* were harvested in Antananarivo in October 2013. They were cut into small pieces before drying in aerated and covered area and grinding. The taxonomic botany of the plant is identified and authenticated by Mr

Rakotonirina Benja, a botanist of IMRA and a specimen voucher is deposited at the IMRA Department of Botany under the CON02/LPA identity.

2.2 Bio-guided extraction and isolation

Five hundred g of plant powder are extracted by maceration with ethanol (90°) during 24 hours, in room temperature with intermittent shaking. The extraction process was repeated 3 times and all the alcohol extracts were gathered. The combined ethanol solutions was depigmented by passage through an activated charcoal layer, filtered on the whatman's filter paper and evaporated to dryness under reduced pressure. This depigmented crude extract was used for the pharmacological study which consists to evaluate its effect on phenylephrine pre-contracted isolated rat aorta.
Then, 40g of this depigmented ethanol extract was partitioned by the liquid-liquid separation method using increasing polarity solvents successively between distillated water (DW)-dichloromethane (DCM), DW-ethyl acetate (AcOEt) and DW-butanol (BuOH). Each fraction was pharmacologically tested and their vaso-relaxing effect was compared to that of the crude extract.

The diagram in the figure 1 shows all used process for the isolation and the purification of the major compound of the butanol fraction. Two serials of column chromatography were prepared using a silica gel (pore size 60Å, 40-60 µm, high purity, FLUKA) as a stationary phase. For the first one, mixtures of solvents were used in increasing polarity regime. We have started with DCM-AcOEt-BuOH (9/10.5/0.5; v/v/v) and we progressively reduced the DCM and AcOEt rates to have 100% of BuOH, followed by a mixture of methanol and BuOH (50-50; v/v) and the elution was finished with 100% of methanol. Fifteen fractions were obtained for this first column chromatography. The 3rd and 4th fractions were gathered and called F34. It was fractioned on column chromatography using the same silica gel as a stationary phase. Compounds were eluted with a mixture of DCM-BuOH (18-2; v/v at the beginning to 100% of BuOH) followed by 100% of methanol. Six fractions were obtained. The major molecule of the 4th fraction was purified using a preparative chromatography on Thin Layer Chromatography plate eluted by a mixture of DCM-BuOH (17-3; v/v). Its purity was verified by ultra-performance liquid chromatography (UPLC) using a mixture of acetonitrile and acidified water as a mobile phase (90/10; v/v).

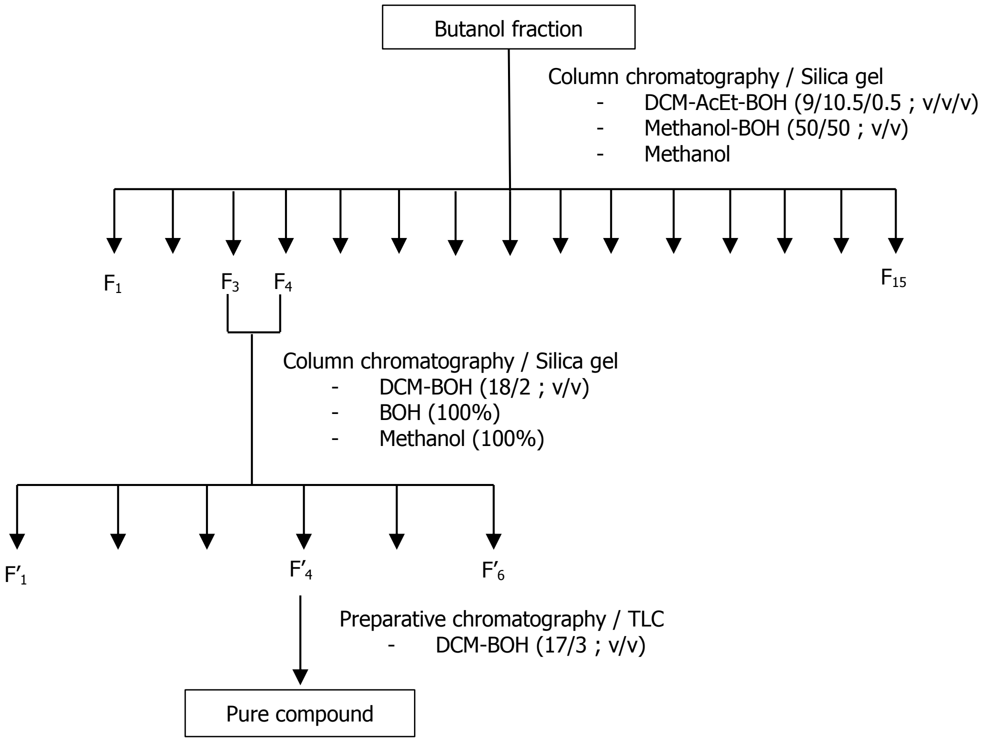

Figure 1: The figure presents the chemical process for the isolation and purification of the major compound in the butanol fraction of *Crassula ovata* (Mill.) (CRASSULACEAE).

2.3 Structural determination: The isolated and purified compound was sent to the Laboratory of Analytical Chemistry of Bioactive Molecules (University of Strasbourg), France, for the chemical structure determination. For this purpose, several physical methods were used including mass spectrometry (UPLC-ESI negative mode-Agilent 6520 Q-TOF), ultraviolet-visible spectrophotometry (UPLC-UV-DAD), nuclear magnetic resonance spectroscopy (500 MHz Bruker NMR spectrometer), and Chemdraw Ultra 11 software.

2.4 Biological test: Ethanol crude extract, dichloromethane and ethyl acetate fractions were prepared in dimethyl-sulfoxide (DMSO). Then, serials of dilution were made in DW to reduce the rate of DMSO in the organ bath to less than 0.25%. While, the butanol fraction, the aqueous fraction and the purified compound were diluted in DW.

The pharmacological effect of the tested products was evaluated on the phenylephrine (PE) pre-contracted isolated rat aorta as previously described by Duarté et al. (1993) [7]. Practically, after equilibration and stimulation, the strip maintained in Krebs-Henseleit solution and aerated with a 95% of O_2 and 5% of CO_2 mixture, was pre-contracted with 10^{-7}M of PE. When the plateau of contraction was reached, different concentrations of product were cumulatively tested (25 - 1600µg/ml for the crude extract and fractions and 5 – 320µg/ml for the gallic acid). The EC_{50} of all tested products were calculated by linear regression. It is the concentration that provokes 50% of relaxation of the pre-contracted rat aorta. The amplitude of the contraction provoked by PE is considered as 100%. The contraction or relaxation of the isolated rat thoracic aorta was measured with an Ugobasile 7003 isometric force transducer.

Adult female or male Swiss rat, aged of 3 months, weighing at about 200g were used for this purpose. They were provided by the Malagasy Institute of the Applied Research (IMRA) animal housing.

2.5 Phytochemical screening investigation: The principle chemical classes of secondary metabolites were detected in the leaf extract of *C. ovata* using specific reagents as previously described. The presence of each chemical compound is characterized by coloration and/or a precipitation reaction. Standardly, the following reagents were used to detect the corresponding chemical class:

- Wagner’s, Mayer’s and Dragendorff’s reagents for alkaloids [8-10],
- Willstatter’s (HCl + Mg) and the Bath-smith’s (HCl + heating) reagents for Flavonoids and leucoanthocyanes [9],
- Gelatin (1%) and ferric chloride solution (5%) for Phenolic compounds and tannins [9-10],
- Borntrager’s test using ammoniac solution for anthraquinones [9],
- Liebermann- Buchard test (a mixture of glacial acetic acid and sulphuric acid, 19:1) for triterpenoids and steroidal nuclei [9-10],
- Salkowski’s test using sulphuric acid for unsaturated sterols,
- Badjet Kedde’s test with picric acid for lactonic steroids (positive result give red coloration),
- keller-killani’s test for cardio-tonic glycoside [11],
- Precipitation of polysaccharides was tested using three volumes of ethanol for one volume of decoction fraction,
- Production of persistent suds for saponins [9].

3. RESULTS

3.1 Vaso-relaxing activity of the depigmented ethanol extract, different fractions and the isolated molecule

Ninety two grams of depigmented ethanol crude extract were obtained from 500g of *C. ovata* dried leaves powder (r=18.4%). It provokes a relaxation, in concentration-dependent manner, of the pre-contracted rat aorta.

After liquid-liquid separations in different solvents, the majority of the chemical compounds in this ethanol extract were dissolved in distilled water 62.5%. While, butanol has extracted only 6.5%. The masse and the EC_{50} on the PE pre-contracted rat aorta of each fraction were shown on the table 1.

Table 1: The table presents the result of the depigmented ethanol crude extract repartition and the vaso-relaxing effect of each fraction on the phenylephrine pre-contracted isolated rat aorta (n≥4).

Extract	Masse (g)	EC_{50} (µg/ml)
Depigmented ethanol crude extract	40	247.04 ± 5
Dichloromethane	0.056	Not significant
Ethyl Acetate	0.750	677.2 ± 7
Butanol	2.6	528.7 ± 2
Aqueous	25.2	232.3 ± 3

n: represents the test number of each serial.

After two serials of column chromatography at atmospheric pressure of 2.56g of the butanol fraction and a preparative chromatography, 7.2±0.1mg of the major product were isolated and purified (Figure 1). Its EC_{50} is 38.8±1µg/ml. Its pH is 3.0±0.5 which demonstrated an acid characteristic of this isolated compound.

3.2 Structural determination of the active molecule

Chromatograms obtained from UPLC / MS (Figure 2) and from the UPLC / UV-Visible (Figure 3) show each other a single peak respective at the 2.51 min and 2.34 mm retention times of the active molecule respectively.

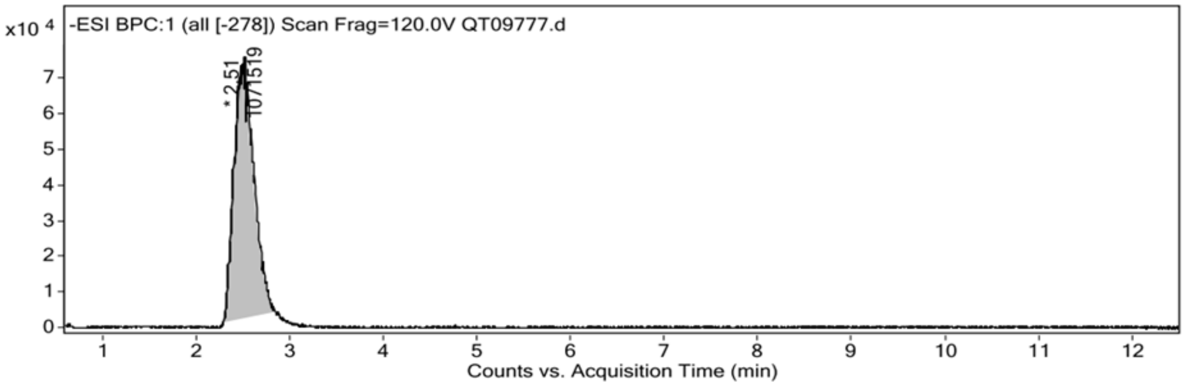

Figure 2: The figure presents the UPLC/SM chromatogram of the active compound.

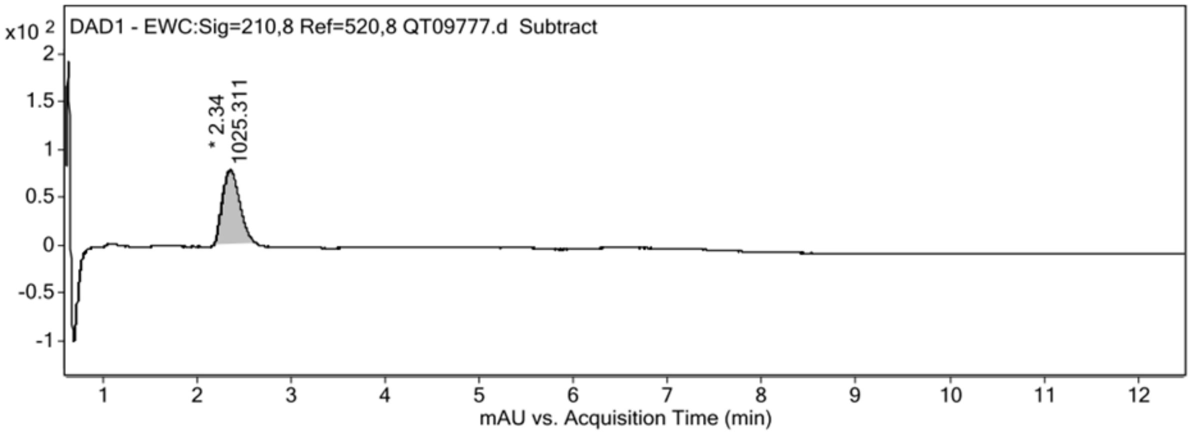

Figure 3: The figure presents the UPLC/UV-Visible chromatogram of the active compound.

The mass spectrum obtained by ESI shows two peaks whose base peak corresponds to m/z 169 and a fragment peak at m/z 125 respectively corresponding to $[M - H]^-$ and to $[M - CO_2 - H]^-$.

The UV-Visible spectrum shows 2 absorption bands at wavelengths 218nm and 272nm.
The ^1H-NMR spectrum shows 3 singlet peaks, 2.51 ppm and 3.17 ppm correspond to the peaks of the solvent (DMSO-D6) and 6.89 ppm for protons of the active compound.

The ^{13}C-NMR spectrum shows 6 singlet peaks, 40 ppm corresponds to DMSO-D6 and peaks at 109.3, 124.2, 137.7 and 145.7 ppm being ethylenic or aromatic carbons and last at 169.8 ppm corresponds to a carboxyl carbon.
The Hetero-nuclear Single Quantum Correlation (HSQC) spectrum shows two ^1H-^{13}C direct correlation spots, the first (2.51; 40) of which corresponds to the solvent and the second (6.89; 109.3) corresponds to protons and aromatic carbons.

Hetero-nuclear Multiple Bond Correlation (HMBC) spectrum of the active molecule shows 5 ^1H-^{13}C correlation spots separated by two or three bonds, including 3 intense spots, medium intensity spot and low intensity spot that are aligned to 6.89 ppm of the proton spectrum.

Table 2: The table presents the values of chemical shifts of carbons and protons and their characteristics.

Spectra	Chemical shift (ppm)	Multiplicity	Type of carbon
Proton	6.89	Singlet	CH
Carbone 13	169.8	Singlet	Quaternary
	145.7	Singlet	Quaternary
	137.7	Singlet	Quaternary
	124.2	Singlet	Quaternary
	109.3	Singlet	CH

3.3. Phytochemical screening

The preliminary phytochemical screening of the leaves of *C. ovata* indicates that leaves of this plant are rich in Leucoanthocyanes, phenolic compounds, tannins and polysaccharides and low concentration of steroids. Alkaloids, flavonoids, quinones, anthraquinones, terpenoids and saponins were conspicuously absent (table 3).

Table 3: The table presents the result of the phytochemical screening of the *C. ovate* ethanol crude extract.

Phytochemical	Result
Alkaloids	-
Flavonoids	-
Leucoanthocyanes	+++
Phenolic compounds	+++
Tannins	+++
Quinones	-
Anthraquinones	-
Terpenoids	-
Steroids	+
Lactonic steroids	-
Unsaturated sterols	-
Cardio-tonic glycosides	-
Polysaccharides	+++
Saponins	-

+++ : High concentration of phytochemical compounds
+ : Low rate of phytochemical compounds
- : Absence of phytochemical compounds

4. DISCUSSION

The ethanol crude extract of *C. ovata* leaves shows an interesting vaso-relaxing activity which could explain, at least partially, its anti-hypertensive virtue. Effectively, the vaso-dilating drugs like the anti-calcic, the inhibitors of angiotensin converting enzyme, the angiotensin II receptor antagonists, the endothelin I receptor antagonists and the nitrate organics constitutes an important antihypertensive therapeutic class [12]. According to the Table 1, the aqueous fraction is the most active fraction. It can indicate that the bio-active metabolite responsible of this vaso-relaxing activity is most probably a polar molecule like a polysaccharide or a phenolic compound non-tannin. Effectively, leaves of *C. ovata* are rich in these two molecular chemical families (Tabl.3). Actually, a bio-guided fractionation on the aqueous fraction is undertaken to identify and isolate the bio-active molecule. While, the vaso-relaxing effect of the butanol fraction is less potent than that of the crude ethanol extract (Tabl.1). This fraction is mostly constituted by gallic acid. It explains the acidity of its pH (3.0±0.5). The gallic acid anti-oxidative activity is well documented [13-17] and can contribute in the benefic therapeutic effect of this medicinal plant in the high blood pressure management. Single peaks obtained on two chromatograms (Fig.2 and 3) confirm the purity of the isolated molecule.

Concerning the structural determination, a pseudo-molecular ion peak at m/z 169 obtained on mass spectrum corresponds to $[M\text{-}H]^-$. Therefore, the molar mass of the active molecule is 170 $g.mol^{-1}$ corresponding to the formula $C_7H_6O_5$. Two UV-Visible absorption bands respectively correspond to the absorption of a carbonyl group and a conjugate system. Singlet peak of ^1H NMR spectrum at 6.89 ppm corresponds to one or more isochronous aromatic protons. Peaks at 109.3, 124.2, 137.7 and 145.7ppm are attributed to ethylenic or aromatic carbons and the last one at 169.8ppm corresponds to a carboxyl carbon. The molecule therefore has an aromatic ring and a carboxyl group. Taking into account the number of unsaturation and/or cycle which is 5, four carbons of the active molecule are isochrones two by two. The HSQC spectrum confirms that the protons are carried by the carbon at 109.3ppm. The table 4 summarizes the interpretation of HMBC spectrum. At this step, there is two possibilities of chemical structure, that of 2,4,6-trihydroxybenzoic acid and that of 3,4,5-trihydroxybenzoic acid.

Table 4: The table represents the $^{1}H - ^{13}C$ correlations and the interpretation of HMBC spectrum.

($^{1}H - ^{13}C$) correlation	Spot intensity	Interpretation
(6.89 – 109.3)	Intense	C is in *meta* of proton
(6.89 – 124.2)	Low	C is in *ortho* or in *para* of proton
(6.89 – 137.7)	Intense	C is in *meta* of proton
(6.89 – 145.9)	Medium	C is in *ortho* or in *para* of proton
(6.89 – 169.8)	Intense	C is in *meta* of proton

According to Table 4, the $^{1}H - ^{13}C$ correlations illustrated in the figure 4 can be deduced.

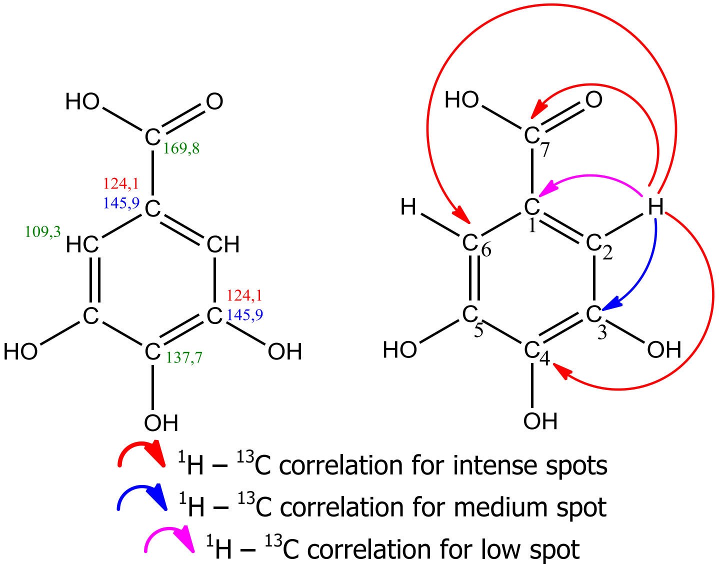

Figure 4 : The figure represents the interpretation of the ^{1}H et ^{13}C correlations in HMBC spetrum

The comparison of the observed ^{13}C chemical shifts and that of the predicted with Chemdraw software (table 5) allows to determine without ambiguity the molecule structure and to identify it as 3,4,5-trihydroxybenzoic acid or gallic acid (figure 5).

Table 5: The table shows the values of the isolated compound ^{13}C chemical shifts and that of the predicted with chemadraw software.

Number of C	Observed values	Predicted values	
		2,4,6-trihydroxybenzoic acid	3,4,5-trihydroxybenzoic acid
1	124.2	97.4	125.6
2	109.3	165.0	110.1
3	145.9	96.1	148.6
4	137.7	166.1	141.2
5	145.9	96.1	148.6
6	109.3	165.0	110.1
7	169.8	169.8	169.8

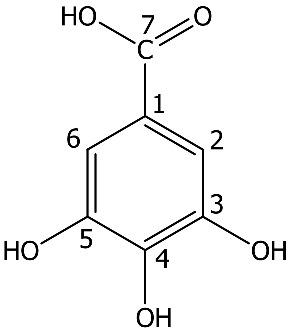

Figure 5: The figure shows the gallic acid structure.

m/z 169 is obtained by chemical ionization of the molecule which lose CO_2 to form the fragment ion at m/z 125 (Fig. 6).

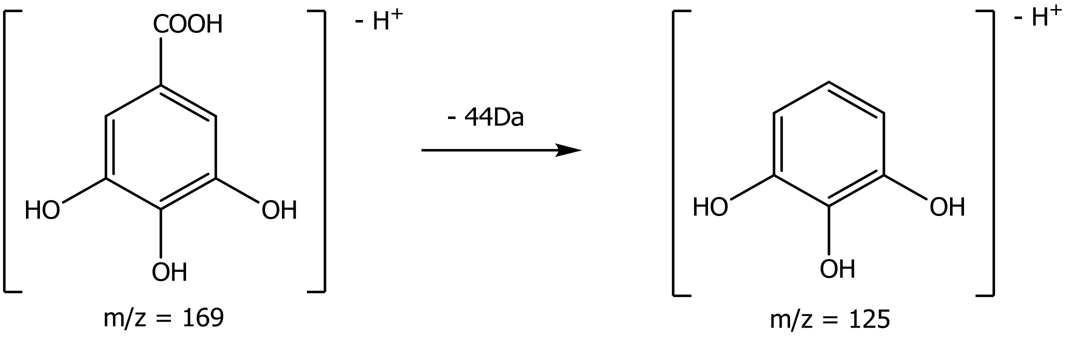

Figure 6: The figure shows the formation of the fragment ion at m/z 125.

Gallic acid is a phenolic compound. Its structure occurs it a source of readily available hydrogen atoms so that radicals produced can be delocalized over the phenolic structure [13]. It has potential preventive and therapeutic effects in many diseases, where the oxidative stress has been implicated, including cardiovascular diseases, cancer, neurodegenerative disorders and in aging [14-15]. Gallic acid has reported its many several pharmacological activities including hepatoprotective activity [18-20], anti-oxidant [16,17,26] and antibacterial activities [17,21], anti-inflammatory activity [22-23], diuretic activity [24], antiviral activity [25], vasodilator activity [26] and antidiabetic activity [27]. Based on those pharmacological activities, we can suggest that gallic acid can also be responsible of the treatment use of *C. ovata* against stomachache and liver injury.

5. CONCLUSION

The ethanol crude extract of the *C. ovata* leaves showed a vaso-relaxing activity which certainly contributes on the traditional anti-hypertensive virtue of this medicinal plant. The most potent vaso-relaxing activity is observed with the aqueous fraction and the identification and the isolation of the bioactive of this fraction is undertaken. Gallic acid constitutes the major compound of its leaf. It was found in the butanol fraction. It exhibited also an interesting vaso-dilator effect on the phenylephrine pre-contracted isolated rat aorta. Our result reports for the first time the isolation of the gallic acid from this plant.

Acknowledgment

This work was carried out at the Laboratory of Applied Chemistry to Natural Substances (LaCASN) and at the Laboratory of Applied Pharmacognosy (LPA) of the Malagasy Institute of Applied Research. Thus, we thank LaCASN team for their assistance as well as the technicians of LPA personally Mr Randriamihanta N. and Randrianatoandro H.Z. We acknowledge also the members of the Laboratory of Analytical Chemistry of Bioactive Molecules, University of Strasbourg, France for the recording of NMR, MS and UV - Visible spectra.

6. REFERENCES

1. Rabarijaona L.M.P.H., Rakotomalala D.P., Rakonirina El-C. J., Rakotoarimanana S. and Randrianasolo O. Prévalence et sévérité de l'hypertension artérielle chez l'adulte en milieu urbain à Antananarivo. *Revue d'Anesthésie-Réanimation et de Médecine d'Urgence*. 2009 : 24-27.
Available : http://rarmu.org/publications/1(4)/full_text/1(4)_24-27.pdf.
2. Wang Z.Q., Guillot D. and López-Pujol J. *Crassula ovata*, a new alien plant for mainland China. *Collectanea Botanica* 2015; 34(9), 1-4.
Available: http://digital.csic.es/bitstream/10261/129733/1/Crassula-ovata-a-new-alien-plant-for-mainland-China-Lopez-Pujol-Jordi-2015.pdf.
3. Gilman E. F. *Crassula Argentea*, Jade plant. UF/IFAS Extension: University of Florida. 1999. Available: https://edis.ifas.ufl.edu/pdffiles/FP/FP15300.pdf.

4. Mitchel B. *Crassula ovata*: Plant of the month, St Andews Botanic Garden. 2013.
Available: http://www.st-andrews.ac.uk/~gdk/stabg_new/poms/images/pom_pdf/feb13.pdf.

5. Atwel B., Downing A. and Downing K. *Crassula Ovata* (Jade Plant, Money Plant) and CAM Photosynthesis. Kirstenbosch National Botanical Garden. 2014. Available: http://www.mq.edu.au/arboretum/Plants/Plant%20of%20the%20week%20-%20Crassula%20ovata%20-%20Jade%20Plant%20-%20and%20CAM%20photosynthesis.docx.pdf.

6. Grisvard P., Chaudun V., Chouard P., Guillaumin A. and Schneiter P. Le bon Jardinier 2 : la maison rustique. Encyclopédie horticole 152$^{\text{ème}}$ édition, Tome II. Paris: 1964.

7. Duarté J., Vizcaino F. P., Utrilla P., Jimenez J., Tamargo J and Zarzuelo A. Vaso-dilatory effects of flavonoids in rat aortic smooth muscle. Structure-activity relationships. *General Pharmacomogy*, 1993; 24 (4): 857-862.
Available : https://www.researchgate.net/publication/223258543_Vasodilatory_effects_of_flavonoids_in_rat_aortic_smooth_muscle_Structure-activity_relationships.

8. Sahira Banu K. and Cathrine L. General Techniques Involved in Phytochemical Analysis. *International Journal of Advanced Research in Chemical Science*. 2015; 2 (4): 25-32. Available: https://www.arcjournals.org/pdfs/ijarcs/v2-i4/5.pdf.

9. Farnsworth N. R. Biological and Phytochemical Screening of Plants. *Journal of pharmaceutical sciences*. 1966; 55 (3): 225-269. Available: http://onlinelibrary.wiley.com/doi/10.1002/jps.2600550302.

10. Harborne J. B. Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman and Hall. *Fakenham Press Limited, Fakenham, Norfolk*. Great Britain, 1973.
Avalaible: https://www.researchgate.net/file.PostFileLoader.html?id=57dc0df6dc332d6c7a2b0e94&assetKey=AS%3A406964409520134%401474039285395.

11. Odeja O., Obi G. Ogwuche C. E., Elemike E. E. and Oderinlo Y. Phytochemical Screening, Antioxidant and Antimicrobial activities of *Senna occidentalis* (L.) leaves Extract. *Clinical Phytoscience*. 2015; 1 (6): 1-6. Available: https://link.springer.com/article/10.1186/s40816-015-0007-y.

12. Taylor D.A. and Abdel-Rahman A. Novel strategies and targets for the management of hypertension. Advances in Pharmacology, 2009; 57: 291-345. Available: http://www.sciencedirect.com/science/article/pii/S1054358908570086.

13. Nikolic K. M. Theoretical study of phenolic antioxidants properties in reaction with oxygen-centered radicals. *Journal of Molecular Structure: THEOCHEM*. 2006; 774: 95-105.
Available: https://www.researchgate.net/publication/239198734_Theoretical_study_of_phenolic_antioxidants_properties_in_reaction_with_oxygen-centered_radicals.

14. Karamaæ M. A., Kosiñska and Pegg R. B. Comparison of radical-scavenging activities of selected phenolic acids. Polish Journal of Food and Nutrition Sciences. 2005; 55 (2): 165-170. Available: https://www.cabdirect.org/abstracts/20073139097.

15. Kaur S., MICHAEL H., Arora S., Harkonen P. L. and Kumar S. The in vitro cytotoxic and apoptotic activity of Triphala -an Indian herbal drug. Journal of Ethnopharmacology. 2005; 97: 15-20. Available: https://www.ncbi.nlm.nih.gov/pubmed/15652269.

16. Ferk F., Chakraborty A., Simic T., Kundi M. and Knasmüller S. Antioxidant and free radical scavenging activities of sumac (Rhus coriaria) and identification of gallic acid as its active principle. *BioMedCental Pharmacology*. 2007; 7: A71. Available: https://link.springer.com/article/10.1186%2F1471-2210-7-S2-A71.

17. Meddour A., Yahia M., Benkiki N. and Ayachi A. Etude de l'activité antioxydante et antibactérienne des extraits d'un ensemble des parties de la fleur du *Capparis spinosa L. Lebanese Sciences Journal*. 2013; 14 (1): 409 – 417. Available: http://lsj.cnrs.edu.lb/wp-content/uploads/2015/12/meddour.pdf.

18. Kanai S. and Okano H. Mechanism of the protective effects of sumac gall extract and gallic acid on the progression of CCl_4-induced acute liver injury in rats. American Journal of Chinese Medicine. 1998; 14 (3): 333-341. Available: https://www.ncbi.nlm.nih.gov/pubmed/9862021.

19. Nabavi S. F., Habtemariam S., Sureda A., Hajizadeh A., Moghaddam, Daglia M. and Nabavi S. N. Hepatoprotective effect of gallic acid isolated from *Peltiphyllum peltatum* against sodium fluoride-induced oxidative stress. *Industrial Crops and Products*. 2013; 44: 50-55.
Available: http://www.sciencedirect.com/science/article/pii/S0926669012005766.

20. Bouasla A., Bouasla I., Boumendjel A., El Feki A. and Messarah A. Hepato-protective Role of Gallic Acid on Sodium Fluoride-Induced Liver Injury in Rats. *International Journal of Pharmaceutical Sciences Review and Research*. 2014; 29(2): 14-18. Available: http://globalresearchonline.net/journalcontents/v29-2/04.pdf.

21. Borges A., Ferreira C., Saavedra M. J. and Simoes M. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microbial drug resistance*. 2013; 19: 256-265. Available: https://www.ncbi.nlm.nih.gov/pubmed/23480526.

22. Kroes B. H., Van Den BERG A. J., Van Ufford H. C. Q., Van Dijk H. and Labadie R. P. Anti-inflammatory activity of gallic acid. *Planta Med*. 1992; 58: 499-504. Available: https://www.ncbi.nlm.nih.gov/pubmed/1336604.

23. Mard S. A., Mojadami S., Farbood Y. and Naseri M. K. G. The anti-inflammatory and anti-apoptotic effects of gallic acid against mucosal inflammation- and erosions-induced by gastric ischemia-reperfusion in rats. *Veterinary Research Forum*. 2015; 6 (4): 305-311.
Available: https://www.ncbi.nlm.nih.gov/pubmed/26973766.

24. Ramya K., Mohandas S. R. and Ashok K. J. Evaluation of diuretic activity of gallic acid in normal rats. *Journal of Scientific and Innovative Research*. 2014; 3 (2): 217-220. Available: http://www.jsirjournal.com/Vol3_Issue2_16.pdf.

25. Kratz J. M., Andrighetti-Fröhner C. R., Kolling D. J., Leal P. C., Cirne-Santos C. C., Yunes R. A., Nunes R. J., Trybala E., Bergström T., Frugulhetti I. C. C. P., Barardi C. R. M. and Simões C. M. O. Anti-HSV-1 and anti-HIV-1 activity of gallic acid and pentyl gallate. *Mem Inst Oswaldo Cruz, Rio de Janeiro*. 2008; 3 (5): 437-442. Available: https://www.ncbi.nlm.nih.gov/pubmed/18797755.

26. Mudnic I., Budimir D., Modun D., Gunjaca G., Generalic I., Skroza D., Katalinic V., Ljubenkov I. and Boban M. Antioxidant and vasodilatory effects of blackberry and grape wines. *Journal of medicinal food*. 2011; 1X (0): 1-7. Available: https://www.ncbi.nlm.nih.gov/pubmed/22082099.

27. Patel S. S. and Goyal R. K. Cardioprotective effects of gallic acid in diabetes induced myocardial dysfunction in rats. *Pharmacognosy Research*. 2011; 3 (4): 239-245. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3249782/.

Cite this article: Tombozara N., Razafindrakoto Z. R., Ramanitrahasimbola D., Razafimahefa Ramilison R. D., Marchioni E. and Rakotondramanana A. D. Isolation of the gallic acid in the butanolic fraction of *Crassula ovata* (Mill.) Druce (CRASSULACEAE) leaves and its vaso-relaxing effect. *Am. J. innov. res. appl. sci.* 2017; 4(5): 200-207.