Research Journal of Chemical Sciences _____ ISSN 2231-606X
Vol. **4(7),** 7-10, July **(2014)** *Res. J. Chem. Sci.*

Microwave Assisted Extraction of Analgesic Compounds of the Root *of Ximenia americana (*Olacaceae*)*

Kenmogne S.B.[1]*, Ngassoum M.[2], Tchatchueng J.B.[2] Vardamides J.C.[1] and Dongmo A.[3]
[1]Laboratory of Chemistry, Faculty of Science, P.O. Box 24157, University of Douala, CAMEROON
[2]Laboratory of Applied Chemistry, P.O.Box.455 ENSAI Ngaoundéré, CAMEROON
[3]Laboratory of Biology, Faculty of Science, P.O. Box 24157, University of Douala, CAMEROON

Available online at: www.isca.in, www.isca.me
Received 13th May 2014, revised 2nd June 2014, accepted 17th June 2014

Abstract

In the present study, roots of Ximenia Americana were selected on the basic of their phytoconstituents profile and an attempt was made to the use of household microwave for extraction of analgesic compound. The extracts dose of 100, 200, 400 mg/kg body weight were evaluated for analgesic activities using acetic acid induced writhing test and hot plate test in rats. From the results, a longer extraction duration time was observed in soxhlet and maceration than in the microwave extraction by a factor of 240 and 900 respectively. In addition, household microwave extraction showed a much higher extraction rate. The yield obtained by soxhlet extraction method was higher than that of microwave and maceration extraction method, however inhibition of pain by analgesic compounds extracts from microwave assisted extraction method was improved by 9% and 22% with respect to soxhlet and maceration extraction method respectively. Futhermore, Microwave extract also increased the reaction time of the rat on the hot plate by 3sec. and 7sec. compared to soxhlet and maceration extract as well. Finally, household microwave appear to be an alternative extraction technique for fast extraction of analgesic compounds from Ximenia americana in the laboratory scale. It requires shorter time, less solvent, higher extraction rate and better product with better yield.

Keywords: Microwave- assisted extraction, *Ximenia americana* root, analgesic activities.

Introduction

Nowadays microwave heating source has demonstrated to be an alternative energy not only in house uses but also in research work[1-4]. This source of energy is rather clean, energy saving, fast and attractive when it comes to extraction of secondary metabolites from plants[5]. Extraction using microwave means compare to traditional maceration and soxhlet method even thought they have the same extraction principle, is rather faster and have a better yield. Under microwave-assisted extraction, the cell plant tissues are easily broken up and the release of biological compounds is facilitated. The other advantage of using microwave extraction is that, the plant components are not decomposed neither oxidized[6]. Extraction method is therefore an important step in analytical chemistry in attempt to recover target compounds. Many parameters affect the yield and biological properties of medicinal plants. To obtain the highest biological activity of plant extract, it is necessary to use a proper extraction method.

Ximenia americana is a small tree which belongs to the family of Olacaceae[7]. From an extensive literature review, it was observed that the *Ximenia americana* is widely used as a popular alternative remedy pain in certain countries in Africa such as Guinea, Ethiopia, Nigeria, Sudan, Cameroon etc. This plant, by using its crude extracts, especially in aqueous and methanolic form could display some biological activities such as antioxidant, analgesic, anticancer etc[8]. From our knowledge, the extraction of phytoconstituents from *Ximenia americana* with microwave-assisted extraction method has not yet been reported.

In the present work, much effort was focused on the extraction of analgesic compounds from the root matrix of *Ximenia americana* by using a household microwave- assisted extraction method. The results of analgesic activities of the extract were compared with those obtained by the conventional solvent extraction methods.

Material and Methods

The roots of *Ximenia americana* used in this study were collected from Ngaoundere (Cameroon, central Africa) forest in April 2012. The identities of plants were verified by comparing the collected specimens with those in the Ngaoundere University. The collected roots were sun dried for seven days, grounded in an electric grinder (IKA-Universalmuhle M20, Germany) and sieved with mechanical shaker analysensieb, Model NFX 11-501, Germany, through a standard set of stainless steel sieves. The particles of 500 µm fraction were collected and used in the experiment. Chemicals were from Sigma-Aldrich and were used as received. Microwave-assisted extraction experiments were performed with a house domestic microwave (2450 MHz, Toshiba, and Tokyo, Japan) with a

variable power up to 1000 watts. To test biological activities rats (20-30g of either sex) fed with rat pellet food and water ad libitum. Animals were first acclimatized during seven days at the laboratory environment before used. *Ximenia americana* root extract were evaluated for analgesic activity in these rats using acetic acid writhing methods.

Extraction procedure: The root of Ximenia Americana (2.5g) was extracted with 50ml of 80% aqueous methanol (b.pt.60-65) by using microwave irradiation (600W, 55ºC). The extraction was carried out for 1min by interrupting the process to avoid bumping. For soxhlet method, extraction of 10g of dried powder was done by using soxhlet apparatus with 200 mL of 80% methanol for 4h and regarding maceration method, 10g of grounded sample in 200 mL of 80% methanol in water was extracted at room temperature for 15h. Extracts were concentrated under reduced pressure dried and stored at 4ºC temperature in an air tight container for further studies. Percentage yield was also calculated.

Acetic Acid Induced Writhing Test: Extract of Ximenia Americana root was evaluated for the analgesic activity by acetic acid induced writhing test[9] (known as chemical test). Briefly, the extract was test at four different rates (100, 200 and 400 mg/kg body weight) and the inhibition produced was compared with the control group. Aspirin (20 mg/kg) was used as standard analgesic agent. Pain sensation was created by the Intraperitoneal injection of acetic acid (0.7%) at dose of 0.1mL/10g of body weight. 5min after the application of acetic acid, the total number of writhing was calculated during 30 min. The percentage of protection was express using the following formula:

(Control group mean - treated group mean) x 100/control group mean[10].

Hot Plate-Induced Pain Test: In this test (known as mechanical test), rats of either sex were placed on the hot plate (Rolex) maintained at 55°C ±0.5°C. The time between placement on the hot plate and occurrence of licking of the paws, shaking or jumping off from the plate was recorded as response latency[11]. Rats with the basal latency of more than 10 sec were not included. The response latencies or reaction time was measured before administration (basal) and at 30 min after administration of the test compound (at the same dose as described earlier) of various extraction methods or standard drug (Tramadol) 20mg/kg, i.p) and compare with control group. A cut-off reaction time was fixed at 20 sec to avoid damage to the paws.

Results and Discussion

Three extraction techniques were compared by evaluating the analgesic activities of *Ximenia americana* root extracts. The experiments were performed on the same sample to solvent ratio (1:20) and biological activities were analyzed on the rats with the same dose of extract (200mg/kg body weight). Comparison of *Ximenia americana* extraction using household microwaves, soxhlet apparatus and maceration are listed in table-1. The yield of soxhlet extract (39.24%) for 4h were higher than that of microwave extracts (37%) for 1 min and that of maceration extract (18.21%) for 15h. Regarding the extraction time, our result show that microwave-assisted extraction appeared to be the fastest method since the extraction could be achieved within 1 min. Whereas Soxhlet and maceration took longer time that is 4 h and 15h respectively. In addition, the quantity of the solvent used in microwave-assisted extraction was less, confirming that, microwave-assisted extraction is really cost effective. Similar results were reported when comparing conventional extraction techniques with microwave-assisted extraction during extraction of saponins from cultured cells of Panax notoginseng[12] and total triterpenoid saponins from *Gonoderma atrum*[13]. More-over microwave irradiation method has shown to be very rapid, reliable and economic for production of Schiff bases[14].

Microwave, soxhlet and maceration extracts of Ximenia Americana were tested and compared for analgesic activities. Ximenia Americana root exhibit a comparable analgesic activities with that of standard Aspirin or Tramadol under the rate of 20 mg/kg body weight tested (table-2 and table-3). The two different methods used (mechanical and chemical) showed that the methanol extract posseses analgesic effect at all dose tested.

Table-1
Comparison of Analgesic activities of *Ximenia americana* root extracts from microwave, soxhlet and maceration extractions by chemical method (acid acetic writing test)

Parameters	Microwave extraction	Soxhlet extraction	Maceration extraction
Sample to solvent ratio	2.5g: 50mL MeOH/H_2O,80:20 vv	10: 200mL MeOH/H_2O, 80:20 vv	10g: 200mL MeOH/H_2O, 80:20 vv
Duration	1min	4h	24h
Temperature (°C)	80	65	Room temperature
Color	Brown	Brown	Brown
%Yield (w/w)	37±0.05	39.24±0.08	18.21±0.05

The experiment was replicated three times and the average values were taken.

Table-2
Comparative evaluations of Analgesic activities of MAE extract with other extraction techniques by chemical method (acid acetic writing test)

Group	Doses mg/kg	Number of writhings	%Inhibition of writhings
Control	-	31.2 ± 3.4	-
Aspirine (Standard)	20	16.6 ± 1.3	46.79±0.19
Maceration extract	100	25.2 ± 3.2	19.23±0.28
	200	16.2 ± 2.2	35.70±0.21
	400	12.5 ± 1.6	59,61±0.32
Soxhlet extract	200	08.4 ± 1.3	73.17±0.31
MAE extract	200	05.8 ± 1.1	81.43±0.25

Values are means ± SD of 5 animals

Table-3
Comparative evaluations of Analgesic activities of MAE extract with other extraction techniques by mechanical method (hot plate test)

Group	Dose (mg/kg)	Reaction time (sec.)
Control	-	1,34 ± 0,69
Tramodol (standard)	20	15,05 ± 0.45
Maceration Extract	100	2.90 ± 0.71
	200	7.77 ± 0.47
	400	5.03 ± 0.66
Soxhlet Extract	200	11.39 ± 0.37
MAE Extract	200	13.93 ± 0.49

Values are means ± SD of 5 animals.

In chemical test, maceration extract showed that the response was dose dependant. The maceration extract (100 mg/kg body weight) showed a percentage inhibition of 19±0.28 whereas at 400 mg/kg, it was rather 59.61±0.32. Aspirin was used as the standard drug for the determination of the analgesic activity by chemical method. Aspirin (20 mg/kg) showed an inhibition percentage of 46.79±0.19. Inhibitions of 81.43±0.25% (200mg/kg body weight) with microwave and 73.17±0.31% (200mg/kg body weight) with soxhlet were obtained. Response from treated group was higher than that of the standard group [20mg/kg, (35.79±0.19%)] (table-2). From these results, it can be emphasized that the analgesic response depends on the extraction method and the dose administrated.

With mechanical method, the reaction time with microwave methanol extract was 13.93 ± 0.49 sec (200mg/kg) which was more effective followed by soxhlet 11.39 ± 0.37sec. (200 mg/kg body weight) and maceration 7.77 ± 0.47 (200 mg/kg body weight) extract. The activity appeared to not dose dependent, since the maceration extract at 200mg/kg gave a reaction time of 7.77 ± 0.47 sec. while at 400 mg/kg, it was 5.03 ± 0.66 seconds (table-3); this different in reaction time might be due to biological variation within animals.

The activity of the 200mg/kg microwave extract was higher than that of 20 mg/kg aspirin. This indicates that microwave methanol extract could be used even at 200kg/mg to compliment Aspirin. All determinations were done in triplicates and the mean values were calculated. The result strongly suggests that microwave treatment is a viable means for extracting valuable analgesic compounds from complex matrices.

In convention (soxhlet) the heat transfer and the mass transfer are limiting factors compare to microwave heating, where heat transfer occurs from the centre of samples to the outer colder environment, and volumetric heating effect leads to a faster rise in temperature. Moreover, the internal heating of *in situ* water within the plant material distends the plant cells and leads to the rupture of the glands and analgesic compounds receptable[15]. This might be the reason for the higher extraction of analgesic compounds from microwave extraction than that of soxhlet and maceration extracts. From theses results, household microwave extraction represents a promising alternative method for extracting analgesic compound from natural substrate.

Conclusion

In this study we compared the conventional and microwave assisted extraction methods. Results show that the inhibition percentage of analgesic compounds from *Ximenia americana* obtained by microwave method was improved as compared to conventional soxhlet and maceration methods. Therefore, household microwave-assisted extraction appears to be an alternative extraction technique for fast extraction of analgesic compounds from *Ximenia americana* in the laboratory scale. In order to complete this study, we intend to identify various factors affecting microwave-assisted extraction and to optimize extraction conditions.

References

1. Joshi U., Ware L., Upadhye M., Microwave Assisted Extraction of Crude Drugs, *Int. J. Pharma and Bio Sci.*, **(1)** 330-332 **(2010)**

2. Somani R., *et al*. Optimization of Microwave Assisted Synthesis of some Schiff Bases, *Int. J. Chem. Tech. Res.* **2**, 172-179 **(2010)**

3. Jeeva J. and Ramachandramoorthy T. Microwave Assisted Synthesis and Characterisation of Diamagnetic Complexes, *Res. J. Chem. Sci.* **3(9),** 69-76 **(2013)**

4. Kumudini B., Bharat P. and Sharma V. K., Microwave Induced Synthesis and Antimicrobial activities of various substituted Pyrazolidines from Chalcones, *Res. J. Chem. Sci.* **4(2),** 68-74 **(2014)**

5. Tatke P. A., Application of Microwave Assisted Extraction for Fast Extraction of Catechin from Leaves of *Anacardium occidentale* L. (Cashew) Contributory Oral Presentation Proceedings of the International Symposium on Current Status Opportunities in the Aromatic and Medicinal Plants. Central Institute of Medicinal And Aromatics Plants (CIMAP Lucknow,) February 21-24 **(2010)**

6. Mandal V., Mohan Y., Hemalatha S. PHCOG Rev: Review Article Microwave Assisted Extraction an Innovative and Promising Extraction Tool for Medicinal Plant Research, *Pharmacognosy Review* **1**, 7-18 **(2007)**

7. Hutchinson, J., Dalziel, J.M., Flora of West Tropical Africa. T. Nelson and Sons, London. **(1954)**

8. Siddaiah M.,Jayaveera K. N., Mallikarjuna R. P., Ravindra R. K., Yashoda K. Y., Narender R. G., Phytochemical Screening and Analgesic Activity of Methanolic Extract of *Ximenia americana*., *J. of Pharmacy and Chemistry,* **3(1)**, 23-25 **(2009)**

9. Koster R. Anderson M. Beer D.J. Acetic acid for analgesic screening. Fed Proceed **18**, 412-417 **(1959)**

10. Dongmo A.B., Ngueefack T.B., Lacaille-Doubois M.A. Antinociceptive and anti-Inflammatory Activities of *Acacia pennata* Wild (Mimosaceae), *J. Ethnopharmacol,* **98**, 201–206 **(2005)**

11. Lanhers M.C., Fleurentin J., Dorfman P., Analgesic, Antipyretic and Anti-Inflammatory Properties of *Euphorbia hirta*. *Planta Med.,* **57**, 225–231 **(1991)**

12. Vongsangnak W., Gua J., Chauvatcharin S. and Zhong. J.J., Toward Efficient Extraction of Notogingseng Saponins from Cultured Cells of *Notoginseng*, *Biochem. Eng. J.*, **18**, 115-120 (**2004**).

13. Chen Y. Microwave Extraction of Total Triterpenoid Saponins from *Gonoderma atrum, J. Food Eng.,* **81**, 162-170 (**2007**).

14. Savalia R.V., Patel A.P., Trivedi P.T., Gohel H.R. and Khetani D.B., Rapid and Economic Synthesis of Schiff Base of Salicylaldehyde by Microwave Irradiation, *Res. J. Chem. Sci.* **3(10),** 69-76 **(2013)**

15. Tatke P., Jaiswal Y., An overview of Microwave Assisted Extraction and its Applications in Herbal Drugs Research, *Res J. Med. Plant,* **5(1),** 21-31 **(2011)**