Chemistry and Materials Research
ISSN 2224- 3224 (Print) ISSN 2225- 0956 (Online)
Vol.6 No.6, 2014

Determination of Anti-schistosomal Finger Profiles of *Chenopodium ambrosoide* Crude Extracts *in* BALB/c Mice Using Thin Layer Chromatography (TLC)

Joseph M. Moilo[1*], Joseph M. Keriko[1], Dorcas S. Yole[2, 3] and Gerald M. Mkoji[4].
[1]Jomo Kenyatta University of Agriculture and Technology, P.O. Box 6200- 00200. Nairobi, Kenya.
[2] Institute of Primate Research (IPR), P.O. Box 24481- 00502, Nairobi, Kenya.
[3]The Technical University of Kenya (TUK), P.O. Box 5242800-00200, Nairobi, Kenya.
[4] Kenya Medical Reseach Institute (IPR), P.O.Box 54840- 00200, Nairobi, Kenya.
*Corresponding author email: josephmoilomunyiri@yahoo.com

Abstract
Plants may contain ingredients that have anti-parasitic activity against parasites of medical siginificance. *Chenopodium ambrosoides* (Wormseed) a wide spread herb in the Family: Chenopodiacea was investigated for anti-schistosomal activity using, the human trematode parasite, *Schistosoma mansoni*, as the target. The plant is well known for its vermifuge and anti-helminthetic properties. The root, stem, leaves and fruit of the plant were extracted sequentially using *n*-hexane, dichloromethane, methanol and distilled water as solvents and tested for anti-schistosomal activity. TLC finger profiles mobile of *C. ambrosoides* extracts showed aqueous (leaf) extract had more R_f spots than methanol (fruit) extract but they were not significantly different ($P > 0.05$). The results of this study suggest that *Chenopodium ambrosoides* aqueous (leaf) and methanol (fruit) extracts has remarkable anti-schistosomal properties, and should be investigated to determine their toxicity and also tested against other parasites as a source novel anti-parasitic compounds.
Keywords*:* R_f - Mobility Relative to front TLC **-** Thin Layer Chromatography

1. INTRODUCTION

Chromatograpy is a versatile technique for separating chemical compounds in a sample. Thin Layer Chromatograpy is primarily used for identifying chemical components in sample and for preparative purposes. The sample is applied to the layer of absorbent near the edge as a small spot of a solution. The solvent which is at the bottom of the container creeps up the layer of adsorbent, passes over the spot, and, as it continues up effects separation of the material in the spot. TLC is carried out by ascent, in a tank which is paper – lined so that the atmosphere inside is saturated with solvent as mobile phase. Detection of compounds on TLC plates is normally carried out by spraying with Vanillin reagent which is heated for 10 min at 110ºC. The plates are observed under 254 and 366 nm uv wavelength. Both methanol (fruit) and aqueous (leaf) crude extracts of *Chenopodium ambrosoide* which had indicated some anti-schistosomal activity in BALB/C mice were subjected to this techniques to determine which of the two extracts was better in reduction of schistosome infection.

2. MATERIALS AND METHODS

2.1 Collection of Plants Materials

The plants used for this study were collected from Westlands and Umoja areas of Nairobi city, Kenya which have similar soil texture and climatic conditions. The identity of the plants was established by a recognized taxonomist from the National Museums of Kenya (Herbarium) and Department of Botany, University of Nairobi.

The plants were freshly picked, stored in plastic bags and transported to the laboratory. The plant parts: roots, stem, leaves and fruits were then sorted out and checked for any unfamiliar conditions before separating them into different parts. The plant parts: roots, stem, leaves, and fruits were separated and dried in good air draft for a period of 6 weeks at room temperature (approx. 25^{o}C) until ready for solvent extraction. The respective dried plant parts were crushed into powdery form using Mekon Micromealers Single Phase located at GoK Chemistry laboratory at Jomo Kenyatta University of Agriculture and Technology (JKUAT).

2.2 Extraction of Crude Extracts from Powdered Materials

The ground materials were standardized by passing them through 0.5 mm sieves. The powdered materials from roots, stems, leaves, and fruits were first subjected to extraction by soaking them in *n* – hexane, dichloromethane, methanol and aqueous solvents each for 3 days (72 hr) to elute the non-polar materials. The solutions were then filtered using Whatman's Filter paper (No.1 medium crystalline). The process was repeated three times for each plant part in order to make sure that all non-polar materials were fully eluted. The filtrates were concentrated using Bochi-Rota Evaporator R200 at 40^{o}C. The product was labeled **Extract I.**

The residues from **Extract I** were resoaked in dichloromethane (DCM) to elute the medium polar materials. The process was repeated as in *n* - hexane extraction to constitute **Extract II**.

The residues from **Extract II** were resoaked again using methanol to elute the polar materials. The extraction process repeated as above to constitute **Extract III**.

All the above concentrated filtrates; **Extract I** and **Extract II** were subjected to drying in fume cupboards at 25ºC for several weeks to remove most of the solvents.

The residues from **Extract III** were further re-soaked in distilled water for 3 days (72 hr) in order to extract any remaining polar materials. The resulting solutions were then filtered and subjected to freeze drying using Chemlabs Instrument Ltd (Edwards). The freeze dried materials constituted the aqueous **Extract IV**.

2.3 Detection of Crude Extracts Compounds using Thin Layer Chromatography (TLC)

The plant crude extracts (*n* - hexane, dichloromethane, methanol and aqueous) were subjected to thin layer chromatography (pre-coated silica gel 60 F_{254}, Merck, German). TLC was carried out by ascent, in a tank which was paper – lined so that the atmosphere inside was saturated with dichloromethane as mobile phase. Detection of compounds on TLC plates was carried out by spraying with Vanillin reagent which was heated for 10 min at 110ºC. The plates were observed under the 254 and 366 nm uv wavelength (Min. uvis DESAGA-SARSTEDT.GRUPPE). Spots were outlined and R_f values of the separated fractions were recorded and reported.

3. RESULTS

Methanol (fruit) extract showed that a dilution of dichloromethane : methanol (9:1) eluted two spots with R_f values of 0.362, 0.525; a dilution of dichloromethane : methanol (7: 3) eluted two spots with R_f values of 0.731, 0.902; a dilution of dichloromethane : methanol (3:7) eluted two spots with R_f values of 0.700, 0.925 and a dilution of dichloromethane: methanol (1:9) eluted two spots with R_f values of 0.236, 0.796 and a dilution of dichloromethane : methanol (3 2) eluted four spots with R_f values of 0.171, 0.526, 0.723 and 0.907.
A repeat fractionation of methanol (fruit) extract was done to confirm the number of spots eluted using a dilution of dichloromethane: methanol (3:2). Methanol (fruit) extract showed that only three spots with R_f values of 0.562, 0.725, 0.850 were eluted. The fractionation of methanol (fruit) extract using a dilution (MeOH: Dist.H_2O (1:1) was done. The result showed that no spots were eluted.

The aqueous (leaf) extracts was subjected to fractionation using dichloromethane: methanol dilutions. Aqueous (leaf) extracts showed that using dichloromethane: methanol (9:1) no spots were eluted. Using a dilution of dichloromethane: methanol (7:3) two spots with R_f values of 0.562 and 0.605 were eluted. Using a dilution of dichloromethane: methanol (3:7), one spots with R_f values of 0.787 was eluted. Using a dilution of dichloromethane: methanol (1: 9), two spots with R_f values of 0.162 and 0.637 were eluted and using a dilution of dichloromethane: methanol (3:2), two spots with R_f values of 0.320, 0.705 were eluted.

A repeat fractionation of aqueous (leaf) extract was done to confirm the number of spots eluted using a dilution of dichloromethane: methanol (3:2). The repeat aqueous (leaf) extracts showed that three spots with R_f values of 0.212, 0.562, and 0.687 were eluted. The fractionation of aqueous (leaf) extract using a dilution (MeOH: Dist.H_2O (1:1) was done. The result showed that two spots with R_f values of 0.087 and 0.85 were eluted.

In this study, the results showed that the dilution of dichloromethane: methanol (3:2) eluted more compounds in both the methanol (fruit) and aqueous (leaf) fractions more than other solvents. The fractions from the dilution of dichloromethane : methanol (3:2), were likely to be more efficacious in activity more than the other fractions due to the number of compounds eluted from the plant crude extracts and the profiles might differ depending on the type of solvent used in extraction of the plant crude extracts.

Table 1: The Results of TLC (R_f) Spots from *Chenopodium ambrosoides* Methanol (fruit) and Aqueous (leaf) fraction crude extracts

Dilution ratio of solvents	**No. of spots from Methanol(fruit) Crude Extract (R_f)**	**No. of spots from Aqueous(leaf) Crude Extract (R_f)**
DCM : MeOH (9 : 1)	0.362, 0.525	-
DCM : MeOH (7 : 3)	0.731, 0.902	0.562, 0.605
DCM : MeOH (3 : 7)	0.700, 0.925	0.787
DCM : MeOH (1 : 9)	0.236, 0.796	0.162, 0.637
DCM : MeOH (3 : 2)	0.171, 0.526, 0.907, 0.723	0.320, 0.705
Repeat DCM : MeOH (3 : 2)	0.562, 0.725, 0.850	0.212,0.562,0.687
MeOH : Dist.H_2O (1 : 1)	-	0.087, 0.85

4. DISCUSSION

In the present study, the result showed that methanol (fruit) fraction extract exhibited more spots than aqueous (leaf) extract suggesting that methanol (fruit) was more efficacious against *S. mansoni* parasites.

Several medicinal plants with anthelmintic activity have secondary metabolites like alkaloids and flavonoids (Klicks, 1985). These metabolites are considered the sources of chemicals responsible for wide therapeutic activities of some medicinal plants (Klicks, 1985). Both ethanolic and aqueous crude extracts have been reported to have antiparasitic inhibition (Oguntoye *et al.,* 2008). Although distinct chemical profiles of plants extracts are not known, in general, hydro-alcoholic extracts of plants may contain some non-polar organic chemicals with wide rage of polarity than aqueous extracts (Nisha *et al.;* 2006).

TLC carried out on aerial parts of *Chenopodium ambrosoides*; among the compounds isolated included hydroperoxide was found to be against epimastigotes of *Trypanosoma cruzi* (Kiuchi *et al.,* 2002). Powder and essential oil obtained from leaves of *Chenopodium ambrosoides* were found to protect grains from damage of insects pests such as *Sitophilus zeamais ambrosoides* (Tapondjou *et al.,* 2002).

Ascoridol, a derivative of *Chenopodium ambrosoides* demonstrates that natural products can provide anti-parasitic agents (Klicks, 1985). MacDonald *et al.,* (2004) argues that nematocidal activity of *Chenopodium ambrosoides* infusions are due to hydrophilic components from ascoridole.

Methanolic extracts of some medicinal plants (eg.*Centratherum anthelminticum*,) have been shown to have complete inhibition of worm motility and subsequent motality (Nisha *et al.;* 2006). Eloff, (1998) revealed that the aqueous extracts are better than ethanolic extracts in *in vitro* anthelmintic activity. Ellof, (1998) urgues that the active principle that reduces anthelmintic activity might be due to different classes of chemicals in the plant parts. These natural products offer an efficient approach to discovering and optimizing new pharmaceutical agents for disease control (Equale *et al;* 2009).

5. CONCLUSION AND RECOMMENDATION

Active ingredients need to be isolated and characterised and their activity determined individually to establish whether the activity was due to synergistic effects between several structurally compounds or due to individual compound.

In order to ensure the authenticity, quality and efficacy of the extracts used above, it is necessary to process to final products which was not done during this stage of the study.

ACKNOWLEDGEMENT

I sincerely thank Messrs S. Kisara**,** Simon Mathenge, Francis Kamau and Francis Nyaga for their technical support during the course of this research work.

REFERENCES

Ellof, J. N. (1998). Which extractant should be used for screening and isolation of anti microbial components from plants? J. Ethanophamacol. 60: 1 – 8.

Kiuchi, F; Itano,Y; Uchiyama, N; Honda, G; Tsubouchi, A; Shimada, J. N and Aoki, T. (2002). Moneterpene Hydroperoxide with Trypanocidal Activity from *Chenopodium ambrosoides*. J. Nat. Prod. 65: 509 - 512.

Kliks, M. M. (1985). Studies on the traditional herbal antihelminth *Chenopodium ambrosoides* L.: ethonopharmacological evaluation and clinical field trials. Soc. Sci. Med. 21: 879 – 886.

McDonnald, D; Vancrey, K; Harrison, P; Rangachari, P. K; Rosenfield, J; Warren, C and Sorger, G. (2004). Ascaridole-fewer infusions of *Chenopodium ambrosoides* contain a nematocide (s) that is / are not toxic to smooth muscles. Journal of Ethanopharmacol. 92: 215 - 221.

Oguntoye, S. O; Olatunji, G. A; Kolawole, O. M and Enonbun, K. I. (2008). Phytochemical Screening and anti-bacterial activity of *Viscum Album* (Mistotle) Extracts. Plant Sciences Reseach. 1: 44 – 46.

Nisha, M; Kalyanasundoram, M; Paily, K. P; Abidba, V. P and Balaraman, K. (2006). *In vitro* screening of medicinal plants extracts for microfilaridal activity. Journal Parasitology Research. 100: 575 - 579.

Tapondjou, L. A; Adler, C; Bouda, H and Fontem, D. A. (2002). Efficacy of powder and essential oil of *Chenopodium ambrosoides* leaves as post-harvest grain protectants against six-stored product beatles. Journal of Stored Product Reseach 38: 395 - 402.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar