

Optimization of Extraction Conditions and Phytochemical Screening of Root Extract of *Synadenium glaucescens* Pax

Faith P. Mabiki^{1,3}, Joseph J. Magadula², Robinson H. Mdegela³ & Resto D. Mosha³

¹ Faculty of Science, Sokoine University of Agriculture, Morogoro, Tanzania

² Institute of Traditional Medicine, University of Health and Allied Sciences, Dar es salaam, Tanzania

³ Faculty of Veterinary Medicine, Sokoine University of Agriculture, Morogoro, Tanzania

Correspondence: Faith P. Mabiki, Department of Physical Sciences, Faculty of Science, Sokoine University of Agriculture, P. O. Box 3038, Morogoro, Tanzania. Tel: 255-23-260-3404. E-mail: fmabiki@gmail.com, fmabiki@suanet.ac.tz

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Abstract

Optimization of extraction conditions and phytochemical screening of the root bark of *Synadenium glaucescens* were carried out in a stepwise manner in order to obtain the highest yields and the constituents of the extracts. Sequential extraction using Soxhlet method was performed using dichloromethane, hexane and petroleum ether, respectively, each followed by ethanol. Extraction conditions included: running time of 2 to 6 hours, temperature at 25 °C to 95 °C and particle size ranging from 0.4mm to >3mm diameter. Phytochemical screening was done using derivatisation techniques, gas chromatography-mass spectrometry and high performance liquid chromatography. Extraction with dichloromethane followed by ethanol resulted in a higher yield by 25%, within 4 hrs of extraction, particle size of 1mm, at temperatures of 30 °C for dichloromethane and 75 °C for ethanol. Fatty acid analysis indicated absence of free fatty acids in both Dichloromethane and ethanolic extracts. Silylation and Thin Layer Chromatography indicated the presence of non hindered and hindered functionality and the presence of triterpenoids in the dichloromethane extract. Phytochemical screening of the dichloromethane extracts indicated that it is composed of two main triterpenoids that best matched with Lanosterol (42%) and Cycloartenol (31%). Other minor compounds identified through chromatographic analysis were phytol, ergostadiol, hentriacontane, sitastriol aceate, lupeol and hopenone. The ethanolic extracts indicated the presence of polyphenolic compounds.

Keywords: HPLC, GCMS, soxhlet extraction, phytochemical screening

1. Introduction

The members of the Euphorbiaceae family are widely utilized for different purposes in the world (Mwine & Damme, 2011). The genus *Synadenium* is indigenous to East Africa (Dev & Koul, 1997). The species *Synadenium glaucescens* (*Mvunjakongwa* in Swahili, *Liyugi* in Bena language) are found growing in several regions in Tanzania and indigenous people have been using them for treatment of both animal and human illnesses. A juice made from freshly-crushed leaves is usually ingested for treatment of excessive menstruation (Chhabra, Uiso & Mshiu, 1984). A leaf decoction with lime juice, baking soda and honey added is ingested to treat asthma; the ashes of dried leaves are mixed with water and applied to the skin to treat leprosy (Schmelzer, Gurib-Fakim, Arroo, Bosch, de Ruijter, & Simmonds, 2008). A root bark extract is taken with sugar to treat a severe cough or tuberculosis or taken as an ear drop to treat an ear ache (Schmelzer et al., 2008). The same species is also used to control poultry diseases such as Newcastle disease (Wickaman, Mbaga, Madadi, & Byamungu, 2006). The latex is used as a fish poison and water extracts of the leaves have demonstrated antimolluscidal activity against *Biomphalaria pfeifferi* (Kloos, Thiongo, Ouma, & Butterworth, 1987; Neuwinger, 2004).

Water is a common traditional solvent used during ethnomedical extractions. However, technical difficulties exist in the isolation of pharmacologically active compounds from aqueous extracts. The aqueous extract of the leaves and stems of *S. glaucescens* is reported to have a positive reaction for tannins, triterpenoids and coumarins while the methanol extract has steroids, triterpenes and anthocyanins and the petroleum ether extract contains carotenoids, steroids, triterpenoids, volatile oils and glucosides (Neuwinger 1994; Rukunga, Gunnar, & Kofi-Tsekpo, 1990). The extracts prepared from the roots of *S. glaucescens* are used in oral administration for

animal and human treatments. However, the yield during preparation and dosing and the chemical constituents are usually unknown. The work reported here aimed to stepwise optimize the extraction conditions to obtain higher yields and to screen the chemical constituents of the root bark of *S. glaucescens*. The work involved sequential extraction of the root bark of *S. glaucescens* using three solvents as a less polar option followed by ethanol as a highly polar solvent for each. The phytochemical screening was done using gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC-UV). Identification of compounds was done by comparing the Kovat's index and computer data base of the National Institute for Standard and Technology (NIST) library.

2. Methodology

2.1 Plant Collection and Pre-Treatment

With the help of a botanist and local informants, fresh root bark was collected from the field at altitudes between 1650m and 1950m above sea level located between 08°34' to 08°49' S and 034°55' to 035°10' E in Njombe district, Njombe region in the southern highlands of Tanzania. The collected plant parts were air dried in an open space (28-30 °C) for 5 days. To avoid any chemical decomposition, dried plant materials were stored in a cool, dry place and pulverized into smaller particles prior to extraction.

2.2 Chemicals

All chemicals used in this study were purchased from Sigma or Fisher Scientific through Sokoine University of Agriculture, Tanzania and the University of York, UK. The chemicals included Hexane (H) Petroleum Ether (PE), Dichloromethane (DCM), Ethanol (Et), *N*-trimethylsilylimidazole (TMSI), Trimethylchlorosilane (TMCS) and Bistrimethylsilyltrifluoroacetamide (BSTFA).

2.3 Extraction Methods

Soxhlet method was used, in which the dry ground samples were placed into thimbles (33 mm diameter, 80 mm length) and extracted using a common Soxhlet apparatus consisting of a condenser, a Soxhlet chamber, and an extraction flask. Thimbles containing the samples were air-dried at room temperature for 24 hours before adding the next solvent, while the filtrate was evaporated using the rotary evaporator to obtain the dry crude extracts that were measured for yield and phytochemical analysis.

2.4 Determination of Extraction Yield

The yields of the crude extracts were calculated using Equation 1. The percentage yield obtained was used to compare the efficiency of different extraction methods.

$$\%Yield = \frac{\text{Amount (g) of the dry crude extract obtained}}{\text{Amount (g) of the dry sample used}} \times 100 \quad (1)$$

2.5 Optimization of Extraction Conditions

The effects of four main factors, namely solvent, running time, temperature, and particle size of the sample, were investigated to optimize the extraction process for high yields of crude extracts. The process involved sequential extraction of 10 g of pulverized sample with less polar solvent (Hexane, Petroleum ether or Dichloromethane) at similar boiling points followed by ethanol at 60 °C. The extraction temperature varied from 25 °C for less polar solvents to 95 °C for ethanol. Reaction time varied between 2-6 hours while the particle size was fixed between 0.4 mm and particles greater than 3mm size. These parameters were varied at the time maintaining the optimized factors, while the solvent to sample ratio was kept constant in all experiment. Each experiment was repeated at least twice, and the calculated yields were compared.

2.6 Sample Analysis with High Performance Liquid Chromatography (HPLC)

The effect of temperature on ethanolic extracts was studied by dissolving 5 mg of dry ethanolic extract in 1ml of HPLC grade methanol and then filtering with a microfilter with 0.2 µm pore size. The HPLC (Shimadzu 20AD) fitted with an auto sampler and a SPA UV detector at 254 nm was used for analysis. A reversed-phase supelco C-18 column (150 x 4.60 mm and particle size of 5 µm) was used for separation with the column temperature set at 40 °C. The sample injection volume was 1µL and flow rate of 1mL/min; mobile phase: Solvent A: Water; Solvent B: Methanol. The following low gradient elution system was used: 0–8 min, 5% B; 8–20 min, 10%–90% B; 20–27 min, 90%–3% B; 27–30 min 97% B. The detection of phenolic contents was done under the same conditions using an HPLC Model Hewlett Packard 1090 liquid chromatography series II, fitted with the Diode array detector (DAD).

2.7 Derivatisation with *N*-Trimethylsilylimidazole (TMSI)

A 50 g sample was mixed with 1ml pyridine and 0.25 ml of TMSI in a reaction vial. The mixture was then heated at 70 °C for 20 min in a heating block. The resulting liquid was analysed in the GC-MS.

2.8 Derivatization with Trimethylchlorosilane (TMCS) in Bistrimethylsilyltrifluoroacetamide (BSTFA)

A 200 µL of TMCS in 1% BSTFA was mixed with 3mg of plant sample in 100 µl Toluene in a closed reaction vial and left to react at 75 °C for 30 min.

2.9 Fatty Acids Derivatisations

Fresh Sodium methoxide (1N) was prepared by dissolving 40 mg of Sodium Hydroxide in 1 ml methanol. 15 µL of the solution was then added to 5 mg of the crude plant sample into a reaction vial diluted with 1 ml hexane. The mixture was incubated at room temperature while stirring at 300 r/min for 20 min. The resulting solution was analyzed using GC-MS.

2.10 Sample Analysis with Gas Chromatography-Mass Spectrometry (GCMS)

A Perkin-Elmer Clarus 560 GC-MS with an auto-sampler was used. A high temperature, non-polar DB5HT capillary column (30 m × 0.25 mm × 0.25 µm) was used. The auto sampler injection volume was 0.5 µl with a split ratio of 25:1. The oven temperature programme was 60 °C for 1 min, ramped up at 8 °C/min to 360 °C and then held for 10 minutes.

2.11 Kovat's Index Calculations

In order to identify the composition of the crude extracts, two methods were employed: calculation of the Kovat's Index (KI) and the NIST library. KI is commonly used as a method which links retention times on all different columns in order to identify the chemical components of a complex mixture in GC. KI was calculated using the Equation 2, KI of an even C12–C60 alkane standard ASTM[®] 5442 (Aldrich) as shown in Figure 2, was then used in calculating the KI of the unknown GC traces for identification of the components.

$$I = 100 \times \left(n + (N - n) \left(\frac{t_{unknown} - t_n}{t_N - t_n} \right) \right) \quad (2)$$

I = Kovats Index, n = The number of carbons in the smaller linear alkane (standard); N = The number of carbons in the larger linear alkane (standard); t = Retention time.

The NIST library is a database containing a fully-evaluated collection of mass spectra, KI and GC data. The NIST mass spectra search database software as installed in the GCMS equipment was used to identify the best match chemical structures of the different components in the unknown crude samples.

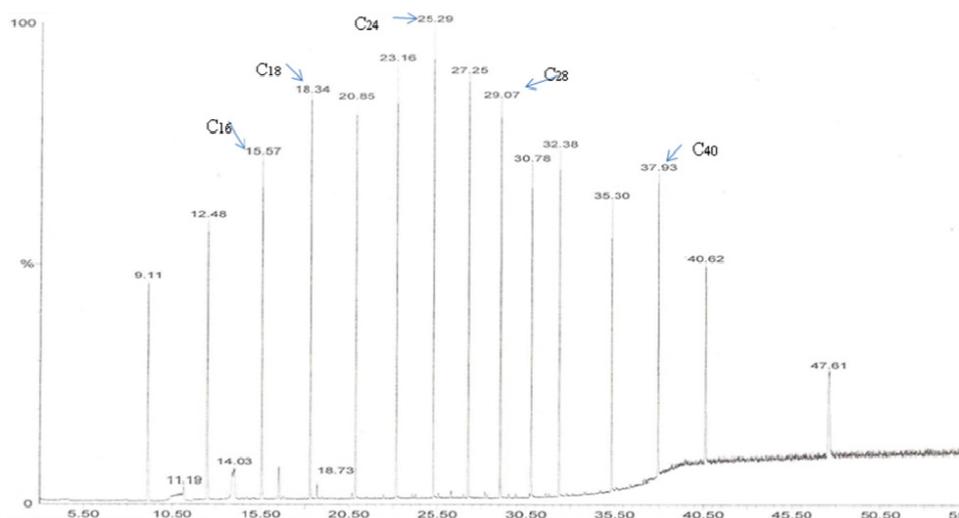


Figure 1. GC traces for a C12-C60 Standard

3. Results and Discussions

3.1 Solvent Optimization

Figure 2 shows the capabilities of each sequential extraction system by percentage. Based on the finding, extraction starting with DCM-ethanol is a better solvent system to attain the highest total yield. The yield obtained by extraction with DCM increased by 6.4% and 4.4% compared to extraction by hexane and petroleum ether, respectively. The total yield of DCM-ethanol extraction was 3.7% higher than the hexane and petroleum ether extraction system. GC traces of hexane and DCM were identical implying that they have similar content. Following the high yields of DCM-Ethanol extraction system, this method was chosen for further optimization studies.

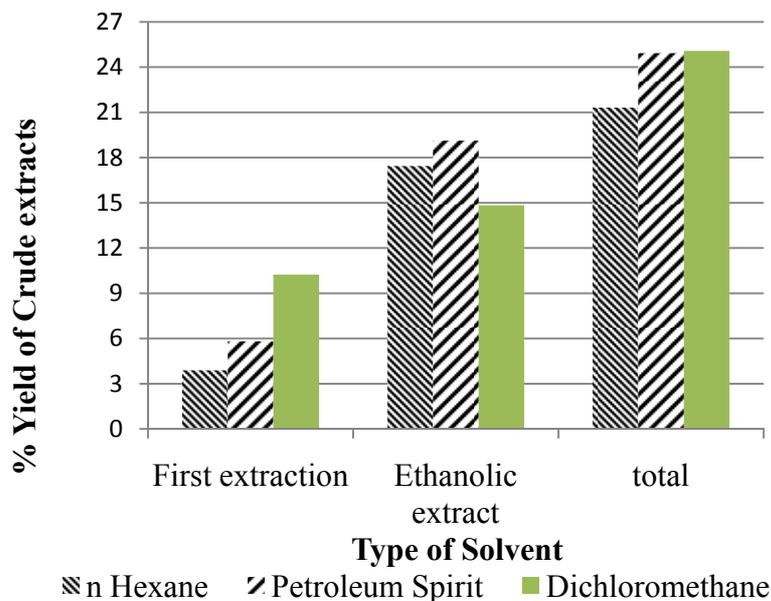


Figure 2. Yields of the three solvent systems at near boiling point with particle size 1.5 mm and running time of 4 hrs

3.2 Effect of Extraction Time

The effect of extraction time is shown in Figure 3. An extraction time of 4 hours demonstrated higher yield for both DCM and ethanol extraction, 10.5% and 14.5% respectively. Obtaining the maximum quantity of extracts at 4 hrs extraction indicates that the root bark of *S. glaucescens* would yield the highest at this extraction duration. Lower yields obtained after 2 hours of extraction indicate that 2 hours was not enough time to extract maximum content from the sample. The decrease of 0.6% yield with 6hrs extraction compared to 4hrs extraction can be explained by either a loss of more volatile components or decomposition due to the long time exposure. This trend could increase with increased exposure time and the nature of the extract composition contrary to the results obtained by Ahmad et al. (2010) during the optimization extraction of herbal Leonuri, showing that extraction with a less polar solvent hexane increased over time from 6 hours to a maximum yield of 7.25% after 12 hours.

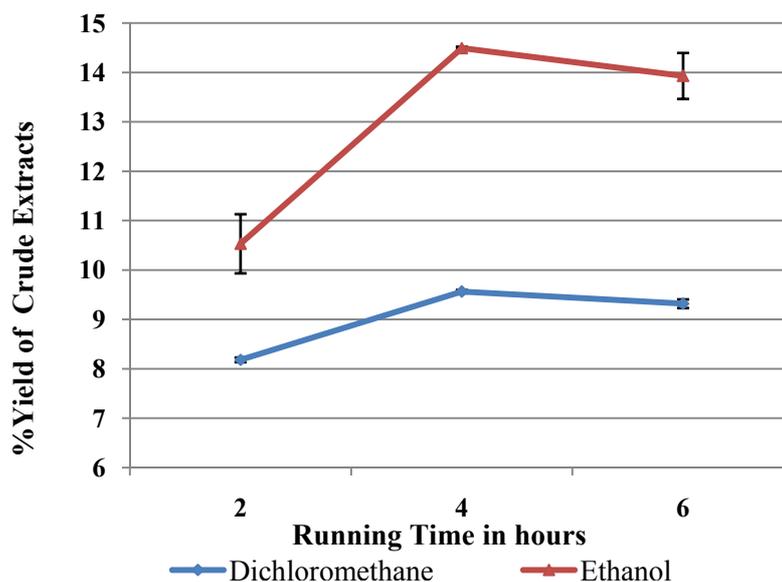


Figure 3. The effect of reaction time on yield of crude extract using dichloromethane- ethanol solvents with the temperature near the boiling point and a particle size of 1.5 mm

3.3 Effects of Extraction Temperature

Figure 4 shows the effect of extraction temperature on yield. Extraction with DCM at 30 °C yielded the highest by 10%, followed by extraction with ethanol which yielded the highest at 75 °C. The yield with DCM increased by 1.1% from 25 °C to 30 °C followed by an inconsistent decrease of 0.1-0.3% to 45 °C. Increasing temperature enhances both the diffusion coefficient and solubility of extracts to the solvent and, hence, improves extraction rate (Richardson et al., 2002). However, for DCM the increase in temperature gave slightly lower yields due to high solvent volatility, reducing the solvent turnover rate. Increasing the temperature from 55 °C to 75 °C with ethanol extraction increased the yield by 9%, after which an increase in temperature by 10 °C resulted in a decrease in yield by 3% which doubled after another 10°C increase. The decreasing yield could be due to the decomposition of some compounds at high temperatures or the evaporation of some volatile compounds from the crude extracts.

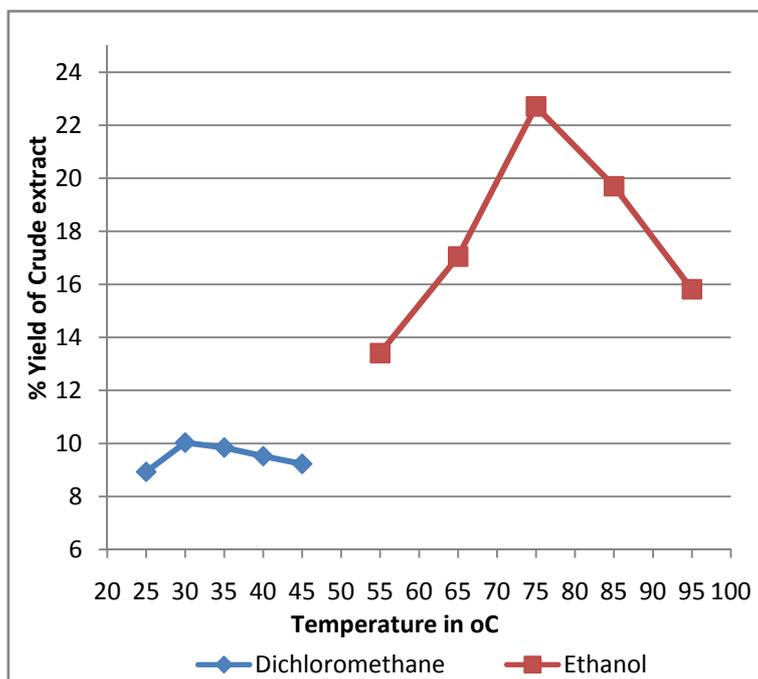


Figure 4. The effect of extraction temperature on yield of extract using dichloromethane-ethanol, running time 4 hrs and particle size 1.5 mm

3.4 Effect of Temperature on Chemical Composition of the Ethanolic Extract Using HPLC

The effect of higher temperature on the chemical composition using HPLC is shown in Figure 5. HPLC analysis indicates some decomposition of compounds at temperatures of 85 °C and 95 °C. This is expressed by a reduction of intensity of some peaks at retention time between 12 and 14min and increased intensities of peaks at retention time between 5.5 and 6.5 min as illustrated in Figure 5.

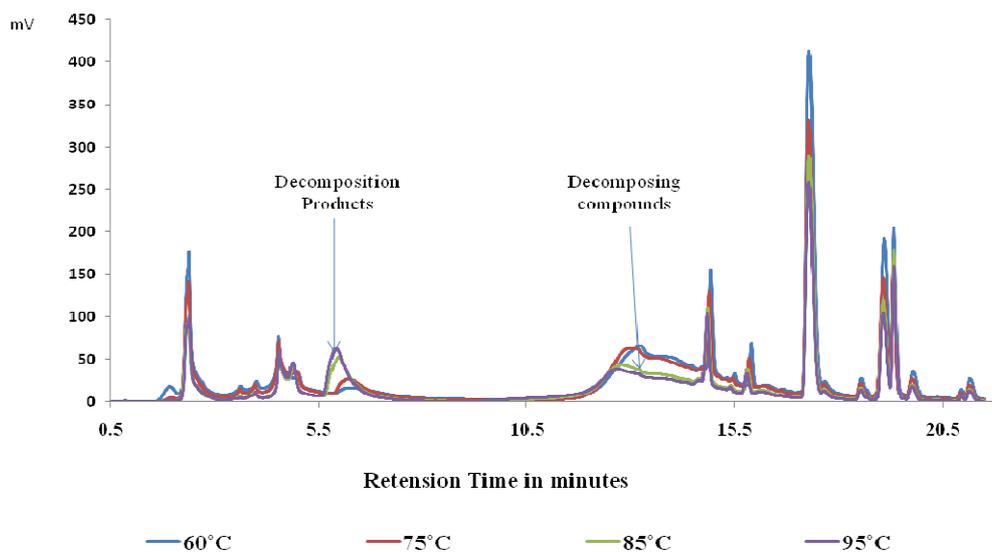


Figure 5. HPLC chromatogram showing the effect of temperature on chemical composition

3.5 Effects of Particle Size of a Sample

Low yields of crude extract were observed in particles larger than 1mm diameter compared to those less than 1mm (Figure 6). For particles greater than 1 mm the yield decreased as the particle size increased; this may possibly be due to the decreased solvent entrance and diffusion of the extract from the particle to the solvent. A smaller particle size offers more surface area for the solvent to diffuse into the sample. This improves interaction between the sample and solvent implying a larger extraction rate and, hence, higher yields. However, when the sample particle is too small agglomeration may occur, which reduces the effective surface area available for diffusion of the solvent in the solid sample (Richardson et al., 2002; Tzia & Liadakis, 2003). The principle holds for a particle size of 0.4mm that yielded less than 2% of the total yield compared to particles with 1mm size. Generally, it can be concluded that a small particle size of 1mm provide a higher yield of crude extracts from the root bark of *S. glaucescens* using Soxhlet extraction. These results are similar to those by Sayyar et al. (2009) who found less than 7% yield with particles of less than 0.5 mm diameter.

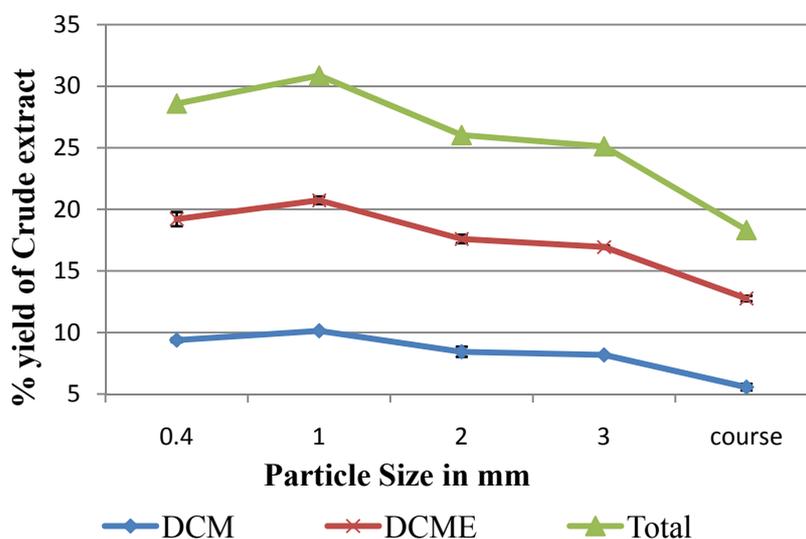


Figure 6. The effect of particle size on yield of extract using dichloromethane - ethanol, running time 4hrs temperature 30 °C and 75 °C for Dichloromethane (DCM) and ethanol (DCME), respectively

3.6 Phytochemical Screening Using GCMS

Fatty acid analysis indicated negative results implying that there were no free fatty acids in the root bark extracts of *S. glaucescens*. Silylation produces trimethylsilyl derivatives which are more volatile, less stable, and more thermally stable. Derivatisation with TMSC mixed with BSA was used for detecting the presence of alcohols, alkaloids, amines and biogenic amines, carboxylic acids, phenols, and steroids. The two reagents indicated the presence of these functional groups in hexane and DCM extracts. Derivatisation with TMSI revealed the presence of hindered hydroxyl groups especially in the ethanol extract of the root. TMSI is a derivatisation reagent which reacts quickly and smoothly with hindered and unhindered hydroxyl and carboxyl groups. TMSI is considered to be the strongest for derivatisation of hydroxyl groups. The GC/MS analysis of hexane and DCM extracts showed an identical composition containing two main triterpenoids of tirucallol or euphol and cyclo euphornol backbone.

Thin layer chromatography analysis at 254 nm indicated a UV negative compound which reacted greenish to deep purple after a colour reaction with vanillin reagent spray and heating. This is an indication of the presence of unconjugated terpenoids and hydroxyl functionality. DCM is reported to have higher potentials of extracting terpenoids and is used specially for this purpose (Tiwari et al., 2011). The dichloromethane extracts were composed of mainly two triterpenoids, see Figure 7. The major compound appeared at RT 32.92 min with calculated KI of 3,273 constituting 42% of the total chemical contents, and it matched best with lanosterol in the NIST library. The second compound appeared at RT 33.3 with calculated KI of 3,326 constituting 31% of the total chemical contents and matched best with cycloartenol in the NIST computer library (Figure 7). The family Euphorbiaceae is characterized with different types of triterpenoids, among which are those with tirucallol, euphol,

cycloephornol and euphorbol backbone (Rizk, 1987). Other minor compounds identified during GCMS analysis include phytol, ergostadiol, hentriacontane, sitastirol acetate, lupeol and hopenone.

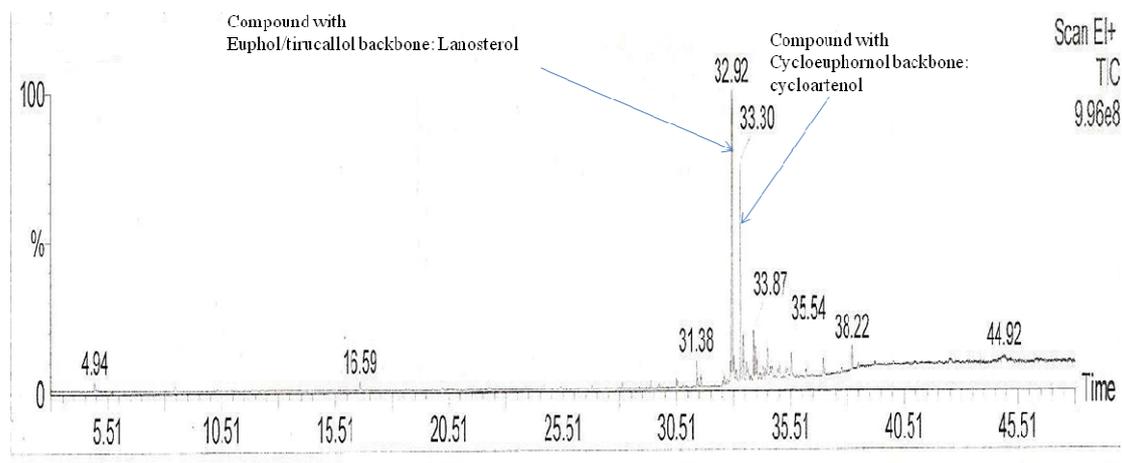


Figure 7. Gas Chromatography traces for hexane extract of the root bark of *Synadenium glaucescens*

The GC traces for ethanolic extract were not significant, thus considered either artefacts or decomposition products of the major product. The ethanolic extract was analysed in HPLC with parameters set for detection of phenolic compounds and showed positive results (Figure 8).

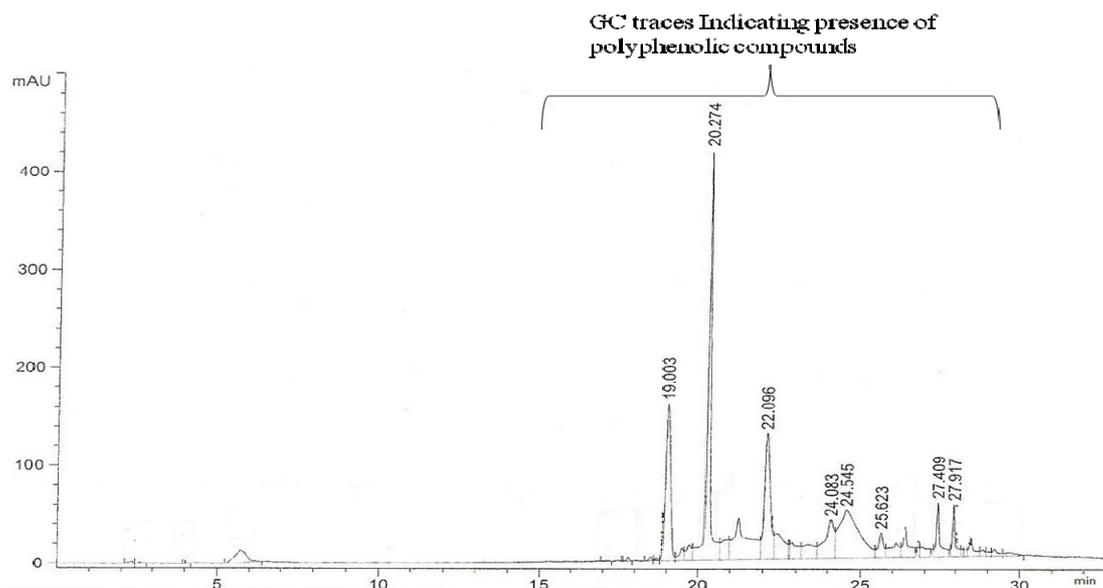


Figure 8. HPLC traces acquired with a reversed-phase gramin C-18 column 150 x 4.60 mm; particle size, 5 μ m; temperature, 40 $^{\circ}$ C; injection volume, 10 μ L, DAD λ , 254 nm and gradient system; specifically set for detection of polyphenolic content in the ethanol extract of the root bark of *Synadenium glaucescens*

4. Conclusion

Four factors affecting the yield from Soxhlet extraction of the root bark of *S. glaucescens* were studied. DCM–ethanol extraction system presented higher extract yields. The optimum condition for extraction was obtained at a running time of 4 hours with a temperature of 30 $^{\circ}$ C for DCM and 75 $^{\circ}$ C for ethanol and a particle size

of 1mm. This study shows that extraction with hexane yields low amounts of extracts. Extraction with petroleum ether yields almost the same amount of extracts with DCM but the mass separation is poor in petroleum ether. Phytochemical screening revealed that the less polar extracts are mainly characterized by triterpenoids while the polar component is composed of polyphenolic compounds. This work should be considered the first information on optimization and screening of organic solvent extracts of *S. glaucescens*. Further studies are recommended to further identify the compounds.

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References

- Ahmad, A., Alkarkhi, A. F. M., Hena, S., Siddique, B. M., & Dur, K. W. (2010). Optimization of Soxhlet extraction of Herba Leonuri using factorial design of experiment. *International Journal Chemistry*, 2, 198-205.
- Chhabra, S. C., Uiso, F. C., & Mshiu, E. N. (1984). Phytochemical screening of Tanzanian medicinal plants. *Journal of Ethnopharmacol*, 11(2), 157-179. [http://dx.doi.org/10.1016/0378-8741\(84\)90037-0](http://dx.doi.org/10.1016/0378-8741(84)90037-0)
- Dev, S., & Koul, O. (1997). *Insecticides of natural origin*. India: Harwood Academic Publishers.
- Farnsworth, N. R. (1988). *Screening plants for new medicines in biodiversity*. Washington D.C.: National Academy Press.
- Kloos, H., Thiongo, F. W., Ouma, J. H., & Butterworth, A. E. (1987). Preliminary evaluation of some wild and cultivated plants for snail control in Machakos district, Kenya. *Journal of Tropical Medical Hygiene*, 90(4), 197-204.
- Mwine, J. T., & Van Damme, P. (2011). Why do Euphorbiaceae tick as medicinal plants? A review of Euphorbiaceae family and its medicinal features. *Journal of Medicinal Plants Research*, 5(5), 652-662.
- Neuwinger, H. D. (1994). *African ethnobotany poisons and drug. Chemistry, pharmacology, toxicology*. Weinheim: Chapman and Hall.
- Neuwinger, H. D. (2004). Plants used for poison fishing in tropical Africa. *Toxicon*, 44, 417-430. <http://dx.doi.org/10.1016/j.toxicon.2004.05.014>
- Richardson, J. F., & Harker, J. H. (2002). *Particle Technology and Separation Processes* (5th ed.). Oxford: Butterworth-Heinemann.
- Rizk, A. F. M. (1987). The chemical constituents and economic plants of the euphorbiaceae. *Botanical Journal of the Linnean Society*, 74, 293-326. <http://dx.doi.org/10.1111/j.1095-8339.1987.tb01052.x>
- Rukunga, M. G., Gunnar, S., & Kofi-Tsekpo, W. M. (1990). Preliminary Chemical Characterization of Pharmacologically Active Compounds of Aqueous Extracts of *Synadenium glaucescens*. *Ancient Science of Life*, 2, 88-93.
- Sayyar, S., Abidin, Z. Z., Yunus, R., & Mohamed, A. (2009). Extraction of oil from Jatropha seeds: optimization and kinetics. *American Journal of Applied Sciences*, 6(7), 1390-1395. <http://dx.doi.org/10.3844/ajassp.2009.1390.1395>
- Schmelzer, G. H., Gurib-Fakim, A., Arroo, R., Bosch, C. H., de Ruijter, A., & Simmonds, M. S. J. (2008). *Plant Resources of Tropical Africa 11(1)–Medicinal Plants 1*. Wageningen: Backhuys Publishers.
- Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Scientia*, 1, 1.
- Tzia, C., & Liadakis, G. (2003). *Extraction optimization in food engineering*. <http://dx.doi.org/10.1201/9780824756185.ch5>
- Wickaman, J. M., Mbagi, T., Madadi, L., & Byamungu, M. (2006). *Assessing Community and Resource Conditions: A Participatory Diagnosis Report for The Baga Watershed Lushoto, Tanzania*.

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