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Chemical characterization of *Syzygium guineense* (Myrtaceae) stem bark extracts

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Abstract
Medicinal plants used in the folk medicine may be an interesting and largely unexplored source for the development of potential new compounds. This study reports on the Phytochemistry and anticancer activity of *Syzygium guineense* (Myrtaceae). *S. guineense* is a medicinal plant that is traditionally used by the Kipsigis and the Ogiek communities in Kenya in the management of various human diseases. Decoctions from the bark of this plant is reported to have been used as a purgative, anthelmintic, antituberculosis, anticancer and treatment of chest ailments. Cold extraction method was used to prepare the crude extracts which were later fractionated and purified using chromatographic techniques (TLC and CC). Two previously established compounds, β-Sitosterol and Betulinic acid, whose anticancer activity has been reported were isolated alongside fatty acids. This study gives a scientific basis for the use of the medicinal plant in the traditional folklore as an anticancer agent.

Keywords: Medicinal plants, anticancer, *Syzygium guineense*, β-Sitosterol and Betulinic acid

Introduction
The chemistry of natural products is very important and can be used in the search for bioactive compounds (Asif, 2015) [2]. Medicinal plants have been used practically in all cultures as a major source of medicament. This usage has been traced to the availability of secondary metabolites with medicinal properties. The availability of medicines plants and their cheaper cost in comparison to modern therapeutic agents makes them more attractive as therapeutic agents (Sharma *et al.* 2010) [18]. Ethno botany and ethno-medicinal studies are recognised as the most viable methods of identifying new medicinal plants or refocusing on those earlier reported for bioactive constituents. Scientific investigations of medicinal plants have been initiated in many places because of their contributions to health care.
The Myrtaceae is a large, well-defined family, with about 140 genera and about 4000 species (Asif, 2015) [2]. The whole family is characterized by leathery glandular leaves containing viscous aromatic terpenoid and polyphenolic substances and flowers with numerous stamens. Several *Syzygium Syzygium* species were reported to possess antibacterial, antifungal, anti-inflammatory and antioxidant activities (Kamsala *et al.* 2014) [10]. The phytochemical studies of this species has revealed that only flavonoids and terpenoids were reported from the leaf and that the plant material has been unexploited much for detailed studies (Pulla Reddy *et al.* 2005) [16].
Syzygium guineense (Myrtaceae) is a small tree with edible fruits (Djoukeng *et al.* 2005) [9]. It is widespread in Sub-saharan Africa where the bark is traditionally used to treat stomachache and diarrhea. This medicinal plant has been used in traditional folk medicine by the Kipsigis and Ogiek communities in Kenya for managing various ailments. However there is unvalidated claim that it has anticancer properties.
The term cancer, malignant neoplasm (neoplasm means new growth) and malignant tumor are synonyms. Cancer is a general term applied to a series of malignant diseases which may affect many parts of the body (Berry *et al.* 2005) [3]. This disease is characterized by a rapid and uncontrolled cell proliferation leading to abnormal growth or tumor. If abnormal growth is not arrested it may progress to death of the patient.
Surgery, radiotherapy and chemotherapy are the options currently available for the treatment of cancer (Chudzik *et al.* 2015) [8]. Chemotherapeutic agents can provide temporary relief but cause serious side effects like bone marrow toxicity, neurotoxicity. Surgery is also not possible in all cases. There is urgent need for effective and safe anticancer drugs. A large number of bioactive compounds exist in various plant species. Among bioactive compounds, an important group is that of triterpenes, which show cytotoxic properties against tumor cells at low activity toward normal cells (Zuco *et al.* 2002) [21]. In our quest for novel bioactive

Secondary metabolites with anticancer properties from plants, chemical characterization of the dichloromethane extract from the bark of *S guineense* was investigated.

Materials and Methods

Sample Collection and preparation

Information of the medicinal plant was obtained through direct interview with the local healers and field observations. Details of the parts used, traditional mode of preparation, route of administration, dosage, duration of the treatment and other plants used together. The bark of a mature S*yzygium guineense* medicinal plant was sampled. Leaves were also collected to aid in authentication. The sample was collected from south west Mau forest, Nakuru County and taken to Chemistry Research Laboratory, Egerton University. The plant identification was performed in the Department of Biological Sciences by Botany specialist. The voucher specimen were labeled with scientific and vernacular names and stored.

The fresh bark was cleaned, chopped to small pieces and air dried inside the research laboratory to avoid direct sunlight that could degrade some of the compounds in the samples. They were then spread out and regularly turned over to avoid fermenting and rotting. This was done for about four weeks till they dried. The dried samples were ground to fine powder using electrical grinder. The powder was then weighed, packed and labeled in sample bags and stored at room temperature.

Isolation and purification

The dried, ground samples were then re-weighed and about 1000 g was soaked in 1.5 L hexane in a 2.5 L bottle for 72 hours at room temperature with frequent shaking. The solvent-containing extracts was then decanted and filtered in a 500 mL beaker through cotton wool to remove course particles and lastly through filter paper (Whatmann No.1) to Obtain crude hexane extract. This was followed by serial extraction using dichloromethane (1.5 L), followed by ethyl acetate (1.5 L) and lastly methanol (1.5 L) in the order of increasing polarity of the solvents for 72 hours each with frequent shaking. It was filtered to obtain the crude extracts in each step. The crude extracts solution were then concentrated under a reduced pressure to a minimum volume using a Rota vapor (Büchi Labortechnik AG, Switzerland). The concentrated crude extracts were allowed to dry to constant weight at room temperature.

The crude extracts were purified using repeated column chromatography and TLC plates. The mobile phase system that was used was hexane/ethyl acetate in the ratio 5:4 and hexane/dichloromethane in the ratio 1:4. The position of visible compounds on the TLC was established by calculating the retardation factor (R_f) which is the distance moved by the compound divided by the distance moved by the solvent. TLC analysis of DCM extract resulted into combining fractions 1-8 (SQ1), 9-18 (SQ2), 20-25 (SQ3) and 27-39 (SQ4) as they had same Rf values. On further purification of SQ1, it resulted in three pure compounds coded SQ6, SQ112 and SQ 111. Similarly further purification of SQ2 resulted in three compounds coded; SQ5, SQ22 and SQ 202 and compounds coded SQ3 and SQ4 were pure. These are summarized in the table below. NMR analysis of the pure compounds was performed on a Bruker 500 MHz NMR spectrophotometer and spectra were recorded in $CDCl_3$ at the University of Surrey. The pure compounds were analyzed using 1D and 2D NMR. Structures of compounds isolated were confirmed by comparison of NMR data against reported literature values.

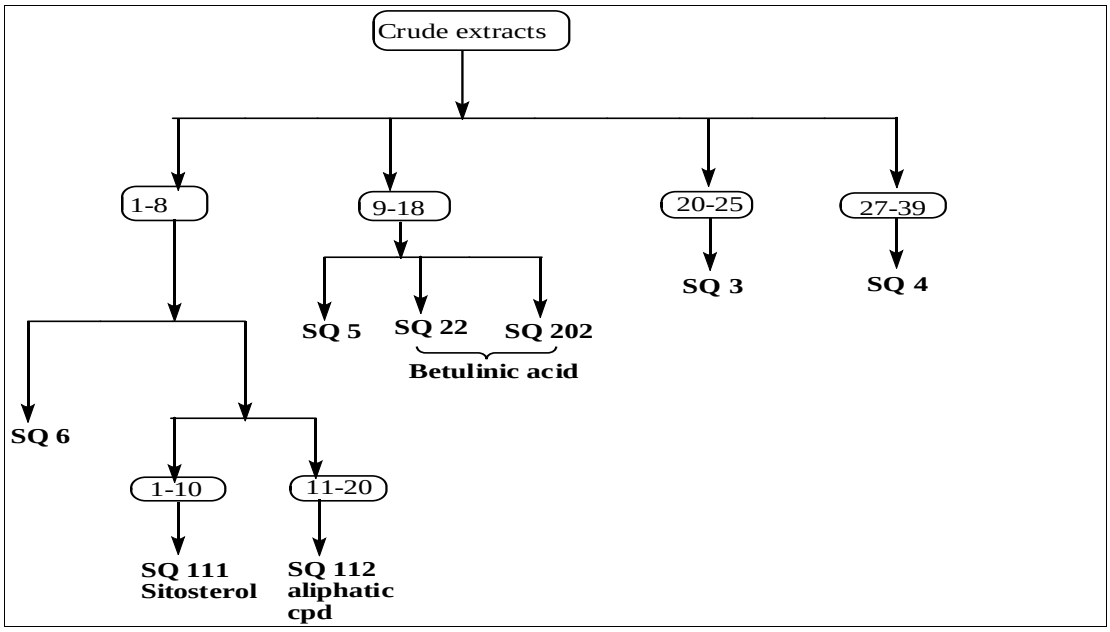


Fig 1: Isolation scheme

Results

Compound SQ 111 was isolated as a white powder. The complete spectral assignments of the isolated compounds were made based on ^{1}H NMR, ^{13}C NMR, COSY, HSQC and HMBC, spectroscopic data. The ^{13}C NMR together with COSY, HMQC and HMBC showed twenty nine carbon signal including six methyls, eleven methylenes, nine methyne and three quaternary carbons. ^{13}C NMR also shows a signal at δ = 140.96 ppm and δ =121.93 ppm for C-5 = C-6 double bond respectively and δ= 72.02 for C-3 β-hydroxyl group.

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of SQ 111 showed the presence of six methyl signals that appeared as two methyl singlets at δ= 0.68, and δ =1.01; three methyl doublets that appeared at δ = 0.81, δ =0.83, and δ =0.93; and a methyl triplet at δ=0.84;. There is evidence of one olefinic proton at δ= 5.36. This is the typical H-6 of the steroidal

skeleton and it appeared as a triplet for one proton. It further showed a proton corresponding to the proton connected to the C-3 hydroxy group which appeared as a triplet of doublet of doublets at δ= 3.53. The position and multiplicity of which was indicative of H-3 of the steroid nucleus. The HSQC shows a correlation between C-6 (δ = 121.93) with a proton at 5.36 ppm, C-3 (δ = 72.02) with a proton at 3.53 ppm and C-13 (δ = 42.53) with proton at 2.30 ppm. On this basis compound SQ 111 was characterized as β-sitosterol (1), the identity of which was confirmed by comparison of the spectral data with previously reported values (Charturvedula and prakash, 2012). These spectral data are summarized in table 1 below. The same approach was used to elucidate the structure of compound SQ 202. The ^{1}H NMR spectrum of compound SQ 202 revealed various peaks corresponding to the methyl groups at around δ= 0.80 to δ = 1.74 ppm and a pair of olefinic protons at δ =4.61 to δ =4.73 ppm, which is characteristic of an exocyclic methylene group. The chemical shift at δ =3.1 ppm was associated with the carbinolic proton attached to carbon 3. The ^{13}C NMR spectrum of this compound (SQ 202) established a lupeol-type triterpene derivative. The characteristic pair of sp^2 carbons comprising the double bond of lupeol was observed as shifts at δ= 150.6 and δ =109.8 ppm. Oxygenated carbon shifts for C-3 was observed at *δ* 79.2. In all, the spectra revealed a compound with thirty carbon atoms (which is equivalent to the total number of carbon atoms in triterpenoid) comprising of six methyl groups, eleven methylenes, six methines, and seven quaternary carbons. The carboxylic acid carbon assigned as C-28 appeared as the most deshielded around 180.2 ppm which is also in agreement with literature. Based on these spectral data the compound was identified as Betulinic acid (2). The structure was confirmed by comparison with literature values (Sharma *et al.* 2010) [18]. The spectral data has been summarized in table 2 below. The structures of the two compounds are shown in figure two below.

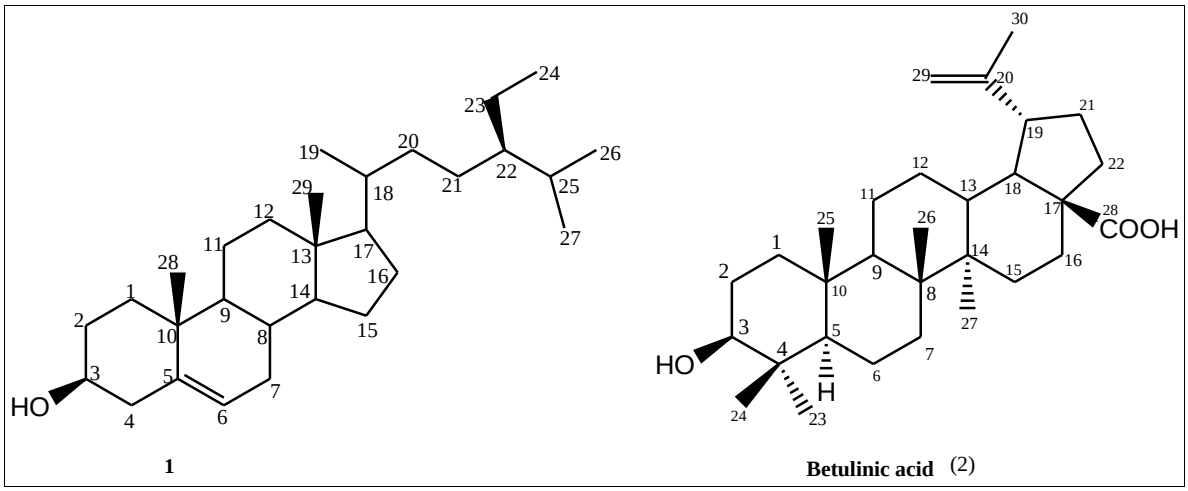
Table 1: NMR spectral data of Compound 1

Position	^{13}C Isolated Compound	^{13}C literature value (Chaturvedula and Prakash, 2012) [6].
1	37.47	37.5
2	31.86	31.9
3	72.02	72
4	42.5	42.5
5	140.96	140.9
6	121.93	121.9
7	32.12	32.1
8	32.12	32.1
9	50.34	50.3
10	36.72	36.7
11	21.3	21.3
12	39.97	39.9
13	42.53	42.6
14	56.98	56.9
15	26.29	26.3
16	28.45	28.5
17	56.27	56.3
18	36.53	36.3
19	19.25	19.2
20	34.16	34.2
21	24.52	26.3
22	46.05	46.1
23	23.28	23.3
24	12.19	12.2
25	29.37	29.4
26	20.02	20.1
27	19.61	19.6
28	18.99	19
29	12.07	12

Table 2: NMR spectral data for Betulinic acid

position	^{13}C Isolated compound (δ)	^{13}C Literature value (δ) (Sharma *et al.* 2010) [18].	DEPT Isolated compound
1	40.2	39	CH_2
2	28.19	27.6	CH_2
3	79.8	78.2	CH
4	39.98	39.1	C
5	57.63	55.5	CH
6	19.6	18.4	CH_2
7	35.7	34.5	CH_2
8	43.7	40.8	C
9	50.6	50.7	CH
10	42.1	37.3	C
11	22.23	21	CH_2
12	27.03	25.7	CH_2

13	39.8	38.1	CH
14	40.2	42.5	C
15	30.9	30.2	CH_2
16	31.85	32.9	CH_2
17	49.7	47.1	C
18	49.6	48.1	CH
19	49.7	49.2	CH
20	152.1	150.1	C
21	31.8	30.6	CH_2
22	38.2	37	CH_2
23	28.1	27.9	CH_3
24	16.25	15.5	CH_3
25	16.7	16.4	CH_3
26	16.87	16.7	CH_3
27	15.25	15	CH_3
28	180.2	180.3	C=O
29	110.3	108.9	CH_2
30	19.59	19.6	CH_3



1 **Betulinic acid** (2)

Fig 2: structures of the isolated compounds

However the structure of the other compounds; SQ3, SQ4, SQ6 and SQ 112 could not be elucidated due to various reasons. SQ3 was found to be a good compound but it was evident from its ^{1}H NMR spectra that it was invested by phthalate thus was abandoned. Similarly SQ4 was found to be invested by fatty acids and therefore nothing much could be done in elucidating its structure. SQ6 was found to be similar to SQ3 and was similarly abandoned. SQ112 was found to be an aliphatic compound. SQ 22 was found to be the same as SQ 202 (Betulinic acid).

Discussion

The compound β-sitosterol (1) is a common chemical constituent of medicinal plants. Phytosterols are made up of a tetracyclic cyclopenta [α] phenanthrene ring and a long flexible side chain at the C- 17 carbon atom. The four rings (A, B, C, D, from left to right) have trans ring junctures, forming a flat α system (Subhadhirasakul and Pechpongs, 2005) [19]. β-Sitosterol and fatty acids from *Mallotus peltatus* leaf extract were reported to show antibacterial and anti-inflammatory activities (Chattopadhyay *et al.* 2002) [5]. B-sitosterol is also known to reduce carcinogen-induced cancer of the colon. It shows antiinflammatory, anti-pyretic, antiarthritic, anti-ulcer, insulin releasing and inhibition of spermatogenesis (Patra *et al.* 2010) [15]. This compound has been isolated from myrtaceae family previously as reported in literature. It was isolated from leaf hexane extract of *Syzigium cumini* and it exhibited antidiabetic effect (Alam *et al.* 2012) [1]. Its isolation was also reported from methanol extracts of *S. cumini* (Sikder *et al.* 2012) [17]. This literature supports the isolation of this compound from *S. guinense*.

The betulinic acid originates from lupane (Chudzik *et al.* 2015) [8]. This is a group of pentacyclic triterpenes, characterized by cytotoxic properties, which may be isolated from plants (e.g., *Spirostachys africana*) (Mathabe *et al.* 2008). or synthetized. Their activity has been proved for cell lines of lung cancer (A549), colorectal carcinoma (DLD-1), breast cancer (MCF-7) and prostate cancer (PC-3), at no activity toward cutaneous fibroblasts (WS1-1) (Chudzik *et al.* 2015) [8]. Triterpenes not only are capable of inhibiting life of neoplastic cell lines, but also induce apoptosis of cancer cells, to cause their "suicidal" death, with no threat to normal cells of the body. Such properties, in particular the selectivity of triterpenes' activity, present them as alternatives in cancer treatment and prevention.

Betulinic acid has been studied extensively owing to its selective antitumor activity against human melanoma cell culture and anti HIV activity (Sharma *et al.* 2010) [18]. It has been reported to inhibit growth of cancer cells, without affecting normal cells and its lack of cytotoxic activity has been demonstrated in human astrocytes, human dermal fibroblasts, peripheral blood lymphoblasts and animal studies (Yang *et al.* 2012) [20]. Betulinic acid has also been reported as catalytic inhibitor of Topo II with IC value of 56.12 μM

comparable to 52.38 μM for a classic Topo II inhibitor etoposide (Moghaddam and Javitt, 2012) [13]. Its isolation from *S. guinense* has been reported by Oladosu *et al.* 2017 [14]. Who studied its anti-tuberculosis effects and found it to be active with an MIC of 0.6 mg/ml. This study supports the availability of Betulinic acid in the *S. guinense* among other chemical constituents.

Anticancer studies of the isolated compounds could not be done since their structures are known and their anticancer properties have been evaluated previously. This study gives the rationale on the ethnobotanical usage of *S. guinense* as anticancer. However toxicological studies need to be done to facilitate its full exploitation as an anticancer agent.

Conclusion

Two compounds were isolated alongside fatty acids from dichloromethane stem bark extract of *S. guinense*. The structures of the isolated compounds were identified as β-Sitosterol and Betulinic acid on the basis of spectroscopic data and comparing their spectroscopic data with those reported in the literature. The anticancer activities of the two compounds have been reported in the previous studies. The result from this study justifies the use of this medicinal plant as anticancer. The authors recommend toxicological studies to be done before this medicinal plant is recommended for use as anticancer.

Conflict of Interest

The authors declare that there is no conflict of interests.

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References

1. Alam M, Rahman AB, Moniruzzaman M, Kadir MF, Haque M, Alvi MR. UH, Ratan MD. Evaluation of antidiabetic phytochemicals in *Syzygium cumini* (L.) Skeels (Family: Myrtaceae). Journal of Applied Pharmaceutical Science. 2012; 2(10):94-98.
2. Asif Mohammad. Bioactive Phytochemical Constituents of Some Edible Fruits of Myrtaceae Family. American Journal of Nutrition Research. 2015; 1:1-17.
3. Berry Donald A, Cronin Kathleen A, Plevritis Sylvia K, Fryback Dennis G, Clarke Lauren, Zelen Marvin *et al.* Feuer Eric J Effect of screening and adjuvant therapy on mortality from breast cancer. New England Journal of Medicine. 2005; 353:1784-1792.
4. Buckingham John. Dictionary of Natural Products, 1998, 4(11).
5. Chattopadhyay Debprasad, Arunachalam G, Mandal Asit B, Sur Tapas K, Mandal Subash C, Bhattacharya SK. Antimicrobial and anti-inflammatory activity of folklore: Mallotus peltatus leaf extract. Journal of Ethnopharmacology. 2002; 82:229-237.
6. Chaturvedula Venkata Sai Prakash, Prakash Indra. Isolation of Stigmasterol, β -Sitosterol from the dichloromethane extract of Rubus suavissimus. International, 2012.
7. Current Pharmaceutical Journal. 2012; 1(9):239-242.
8. Chudzik Malwina, Korzonek-Szlacheta Ilona K Wojciech. Triterpenes as potentially cytotoxic compounds. Molecules. 2015; 20:1610-1625.
9. Djoukeng JD, Abou-Mansour Eliane, Tabacchi Raphael, Tapondjou AL, Bouda H, Lontsi D. Antibacterial triterpenes from Syzygium guineense (Myrtaceae). Journal of Ethnopharmacology. 2005; 101:283-286.
10. Kamsala Ratnam V, Lepakshi Bhakshu Md, Padma Y. Studies on antimicrobial and antioxidant properties of leaf extracts of syzygium alternifolium (WT.) WALP. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; **7**:139-143.
11. Mathabe MC, Hussein AA, Nikolova RV, Basson AE, Meyer JM, Lall N, 2012.
12. Antibacterial activities and cytotoxicity of terpenoids isolated from Spirostachys africana. Journal of ethnopharmacology. 2008; 116(1):194-197.
13. Moghaddam Bita, Javitt Daniel. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. Neuropsychopharmacology. 2012; 37:4-15.
14. Oladosu IA, Lawson L, Aiyelaagbe OO, Emenyonu N, Afieroho OE. Anti-tuberculosis lupane-type isoprenoids from Syzygium guineense Wild DC. (Myrtaceae) stem bark. Future Journal of Pharmaceutical Sciences. 2017 3(2):148-152
15. Patra Arjun, Jha S, Murthy PN, Sharone A. Isolation and characterization of stigmast-5-en-3Î²-ol (Î²-sitosterol) from the leaves of Hygrophila spinosa T. Anders. International Journal of Pharma Sciences and Research. 2010; 1:95-100.
16. Pulla Reddy N, Reddy RV, Narahari, Gunasekhar D. *Chemical constituents of Syzygium alternifolium* (Wt.) Walp. Paper presented at the proceedings of UGC-National seminar on Role of Chemistry in Drug Development Strategies, 2005.
17. Sikder MA, Kaisar MA, Rahman MS, Hasan CM, Al-Rehaily AJ, Rashid MA. Secondary metabolites from seed extracts of *Syzygium cumini* (L.) J Phys Sci. 2012; 23:83-87.
18. Sharma PP, Roy BRK, Gupta Dinesh. Pentacyclic triterpinoids from Betula utilis and Hyptis suaveolens. International Journal of PharmTech Research. 2010; 2:1558-1562.
19. Subhadhirasakul Sanan, Pechpongs Pharkphoom. A terpenoid and two steroids from the flowers of Mammea siamensis. Songklanakarin Journal of Science and Technology. 2005; 27:555-561.
20. Yang Shengjie, Liang Na, Li Hu, Xue Wei, Hu Deyu, Jin Linhong, Zhao Qi and Yang Song.. Design, synthesis and biological evaluation of novel betulinic acid derivatives. Chemistry Central Journal. 2012; 6:1-9.
21. Zuco Valentina, Supino Rosanna, Righetti Sabina C. Cleris Loredana, Marchesi Edoardo, Gambacorti-Passerini Carlo and Formelli Franca.. Selective cytotoxicity of betulinic acid on tumor cell lines, but not on normal cells. Cancer letters. 2002; 175:17-25.