Natural Products Chemistry & Research

ISSN: 2329-6836

Oladunmoye et al., Nat Prod Chem Res 2019, 7:1
DOI: 10.4172/2329-6836.1000356

Research Article Open Access

Identification and Quantification of Bioactive Compounds Present in the Plant *Vernonia amygdalina* Delile using GC-MS Technique

Oladunmoye MK[1], **Afolami OI**[1*], **Oladejo BO**[1], **Amoo IA**[2] **and Osho BI**[3]

[1]*Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria*

[2]*Department of Chemistry, Federal University of Technology, Akure, Ondo State, Nigeria*

[3]*Department of Animal Production and Health, Federal University of Technology, Akure, Ondo State, Nigeria*

[*]**Corresponding author:** Afolami OI, Department of Microbiology, Federal University of Technology, PMB 704, Akure, Ondo State, Nigeria, Tel: +2347069438529; E-mail: afolamiolufemiifeoluwa@gmail.com

Received: January 12, 2019; **Accepted:** February 22, 2019; **Published:** February 27, 2019

Abstract

We conducted structural elucidation studies on organic compounds from the plant *Vernonia amygdalina Delile* using 10% NaOH as extraction solvent. We identified the organic compounds in the plant extract using gas chromatography-mass spectrometry (GC-MS) analysis and we determined the abundance of the compounds present in the plant extract. We also observed the influence of the extraction solvent used (10% NaOH) on the kind of organic compounds recovered from the plant and we juxtaposed the relationship between the most abundant compounds present in the plant extract and their known pharmacological properties. Results showed that organic compounds such as methyl-2-O-benzyl-d-arabinofuranoside, phytol, hexadecanoic acid, ethyl ester, squalene and 9, 12, 15, octadecatrienoic acid are present abundantly (>85% abundance) in the plant extract while other organic compounds characterized in the plant with lesser abundance (<12% abundance) include N-[2-(dimethylamino)-5-pyrimidinyl] benzene sulfonamide, 9, 12, 15 and Octadecatrien-1-ol, p-Menth-4(8)-en-9-ol. The study showed that the extraction solvent used was able to recover compound classes such as organic acid esters and conjugated alkanols in larger quantities than other compound class. The findings of this study demonstrated that *V. amygdalina* contains organic compounds, which may serve as new drug leads of natural products origin and make it employable in modern pharmacological practices.

Keywords: Gas chromatography; Mass spectrometry; Methyl-2-O-benzyl-d-arabinofuranoside; Phytol; Organic acid esters

Introduction

The plant *Vernonia amygdalina Delile* belongs to the family Asteraceae and the common name is "Bitter Leaf" in West Africa [1,2]. Some findings have suggested that the plant is a reservoir for potent phytochemicals of pharmaceutical importance and the plant is of high research interest in southwestern Nigeria [2]. Studies focused on pharmacological significance of local plants always involve structural elucidation of organic compounds the plants might contain to evaluate their therapeutic potentials [3]. There is also a growing interest for precise analyses on quantification of active compounds present in local plants, which necessitates spectrometric studies on these plants to provide good scientific justification for dosage references in prescription studies [3,4].

To achieve thorough study of organic compounds, present in plant materials, the use of rapid analytical methods such as high-performance liquid chromatography (HPLC), Vacuum Liquid Chromatography (VLC) or gas chromatography-mass spectrometry (GC-MS) is necessary [5,6]. However, the GC-MS provides a better holistic approach for characterization of organic compounds present in plant extracts since it combines a rapid separation technique (GC) with a time-dependent identification module (MS) [5]. Accordingly, in this paper, we aim to provide some answers to impending research questions on organic compounds present in the plant *V. amygdalina*. We identified the bioactive compounds present in 10% NaOH extract of *V. amygdalina* using GC-MS and quantified the abundance of the organic compounds elucidated in the plant. We went further to relate the most abundant compounds elucidated in the plant extract with their potentials for use in pharmacological research.

Materials and Methods

Collection and identification of test plant

We harvested dried leaves of the plant *V. amygdalina* from Akure, Ondo State, Nigeria at 7°11' N 7°12' N/5°4' E 5°9' E coordinates. A botanist from the Department of Biological Sciences Plantarium, Federal University of Technology Akure, Nigeria identified the plant *V. amygdalina* used in this study. The identification number assigned was FUTA/BIO/800.

Preparation of plant extract

The solvent used for extraction was 10% sodium hydroxide (NaOH) (Fluka Sweden Grade A). We obtained the powdery form of the plant material prior to the extraction process and adopted the protocols in Huang et al. [7] and Farid et al. [8] to obtain the 10% NaOH extract of the plant. We used a 30 g of the plant into 450 ml of the solvent 10% NaOH at a ratio of 1:15 (powder weight to solvent volume). We obtained the extract at a temperature of 61°C in a Gallenhamp water bath coupled with orbital shaker for 8 hours. We processed the resulting viscous solution after the extraction by filtration using whatman filter paper 1 and we prepared the filtrate separately in 10 ml test tubes. We neutralized the alkali (10% NaOH) in the filtrate with 10

ml each of 0.1 M hydrochloric acid (HCl), centrifuged at 3000 rpm for 10 min and tapped off the supernatant. We partially purified the residue obtained with ice cold 96% Ethanol and centrifuged the mixture again at 3000 rpm for 5 min. Thereafter, we flushed the resulting residue aggregates with distilled water and centrifuged again at 3000 rpm for 5 min to obtain the extract. The weight of extract obtained was 4.54 g.

Derivatization procedure of plant extract before GC-MS analysis

We prepared a stock solution native to the Agilent Turbo Mass Spectrophotometer 7890A GC with chemstation NIST for clarification of the extract. We added 100 µl N, O-Bis (trimethylsilyl) trifluoroacetamide/trimethyl chlorosilane (BSTFA+TMCS) with 20 µl pyridine, we heated the mixture at 60°C for 8 minutes and added 100 µl of ethylbenzene and acetophenone (v/v) to the mixture. We dissolved 1 g of the extract in the standard solution BSFTA+TMCS complex prepared using separate test tubes and added 50 µl acetonitrile. We then filtered the extract using 0.45 µ membrane filter into different Agilent 7890A tube injection vials.

GC-MS characterization of active compounds contained in plant extracts

The authors used Agilent Turbo Mass Spectrophotometer 7890A GC with chemstation NIST for Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The column was Agilent Elite-5 capillary column measuring 30 m × 0.25 mm with film thickness of 0.25 millimeter (mm) and composed of 90% Dimethyl polysiloxane. The carrier gas used was Helium at a flow rate of 0.5 ml/min and we used a 1 µl sample injection volume with an equilibration time of 3 minutes. The inlet temperature was 250°C, oven temperature was initially at 110°C for 2 minutes but increased with a regulated rise to 280°C at a rate of 20°C/min for 9 minutes and the total run time was 38 minutes. The MS transfer line was at a temperature of 315°C and the source temperature was at 350°C. The septum purge flow was 3 ml/min and the total flow rate of the regulated carrier was 11.8 ml/min. The GC-MS was analyzed using electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification. We compared the spectrum of the compounds with database of known compounds stored in the GC-MS library. We used the Turbo-Mass-OCPTVS-Demo SPL software of the NIST Agilent 7890A MS library for measurement of peak areas, organic compound quantification and data processing. We also compared the spectrums of the organic components with the database of literature RI stored in NIMC database spectrum MS-NW-1522. We also juxtaposed the quantities of synonymous organic compounds detected with RI databases such as TSCA, RIECS, and IRDB.

Results and Discussion

The ion chromatogram of the organic compounds elucidated from the plant *V. amygdalina* is in Figure 1. However, the qualitative and quantitative characterization of the compounds contained in the plant extract is in Table 1. Furthermore, we used ChemDraw Ultra 8.0 to sketch the compounds abundantly present in the plant as additional information in Figure 2. The compounds identified from the plant extract include organic acid derivatives, cyclic hydrocarbons, alkanols and polymeric esters. The identities of organic compounds present in the extract suggest that the extraction medium (10% NaOH) is more suitable for recovering of esters and organic acid derivatives from the plant. This affirms previous studies suggesting that alkali extraction medium such as Sodium hydroxide (NaOH) or Potassium hydroxide (KOH) facilitate recovery of polar organic compounds from plants than many other common extraction media [8]. Similarly, a study by Huang et al. [7] reiterated that the use of strong alkali medium for natural products extraction at a temperature below 70°C helps to achieve a faster breakdown of plant cell wall matrix and hence, better dissolution of phyto-compounds. Interestingly, another recent study suggests that the use of hot alkali medium at low concentration for extraction of plant products ensures better yield of phyto-compounds and less damage to compound integrity [9]. In summary, we chose the extraction medium 10% NaOH in our present study to ensure better yield of organic compounds from the plant and to equally discover other compounds in the plant that may not be recovered suppose we used common extraction media such as methanol or ethanol.

As evidenced by our results, the polarity index of the extraction medium and the moderated temperature of extraction helped to recover organic acid esters, conjugated alkanols, nucleoside analogues and a rare class of saturated polyamides; all these compound classes constituted more than 80% abundance in the extract. However, the two most abundant compounds in the plant extract were methyl-2-O-benzyl-d-arabinofuranoside (48.11% abundance) and phytol (20.89% abundance) (Table 1).

Compounds	RT	% Abundance	Quantity (µg/g)	Chemical Formula
Ethyl silyl ether	7.544	0.30	1.93	$C_{12}H_{30}SiO_2$
Phytol	11.448	20.89	134.15	$C_{20}H_{40}O$
Methyl-2-O-benzyl-d-arabinofuranoside	13.430	48.11	308.52	$C_{27}H_{24}O_8$
Butanoic acid ethyl ester	13.757	1.70	10.91	$C_6H_{12}O_2$
Hexadecanoic acid ethyl ester	13.851	6.91	44.37	$C_{15}H_{26}O$
1-Methyl-2-phenylindole	15.077	0.64	4.11	$C_{15}H_{13}N$
Methyl tris (trimethyl siloxy) silane	15.195	0.59	3.79	$C_{10}H_{30}O_3Si_4$
Methyl 8,11,14-heptadecatrienoate	20.210	0.98	6.29	$C_{18}H_{30}O_2$
Squalene	24.062	6.10	39.17	$C_{30}H_{50}$

N-[2-(dimethylamino)-5-pyrimidinyl] benzene sulfonamide	25.048	1.98	12.72	$C_8H_{12}N_2O_2S$
p-Menth-4(8)-en-9-ol	27.626	1.87	12.01	$C_{10}H_{18}O$
2-(4-methyl phenyl)-Indolizene	29.307	1.74	11.17	$C_{15}H_{13}N$
9, 12, 15, Octadecatrienoic acid	30.013	4.90	31.47	$C_{18}H_{30}O_2$
9, 12, 15, Octadecatrien-1-ol	34.736	2.19	14.06	$C_{18}H_{32}O$
Other trace compounds	-	1.10	7.06	-
Total		100	642.18	-
Keys: RT-Retention time in minutes.				

Table 1: Organic compounds in 10% NaOH extract of *V. amygdalina*.

The identity of the compounds in the plant extract showed that the plant *V. amygdalina* contain compound homologues with quaternary structures and organic derivatives which makes the plant useful with bright pharmacological prospects. In a recent review some properties of compounds in the plant *V. amygdalina* was explained [1]. The two most abundant compounds in the extract (methyl-2-O-benzyl-d-arabinofuranoside and phytol) bears similitude with the class of compounds characterized in the study. The compound methyl-2-O-benzyl-d-arabinofuranoside is a conjugated furan-based nucleoside analogue, it exists as a natural product compound of medicinal plants and it was in abundant quantity in the extract. Furthermore, this compound has with antiviral potentials against many enveloped DNA viruses [1]. The second most abundant compound (phytol) is a long chain alkanol with affinity for basal medium. A study by William suggests that many long chain alkanols such as phytol possess antioxidants effects in living hosts [3].

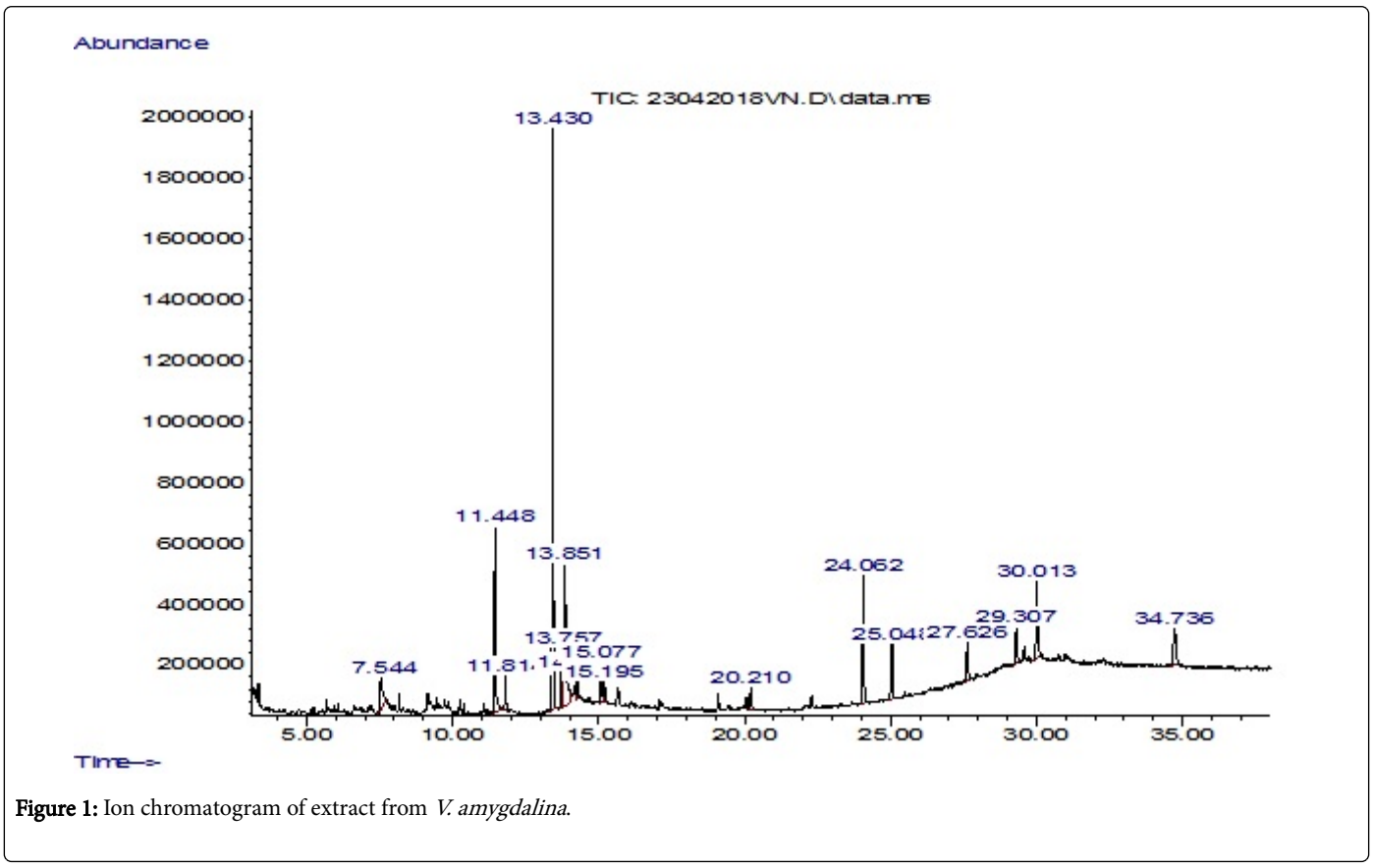

Figure 1: Ion chromatogram of extract from *V. amygdalina*.

Conversely, the other compounds present in the plant extract with <8% abundance are hexadecanoic acid ethyl ester, squalene and 9, 12, 15, octadecatrienoic acid see Table 1. These compounds are mostly common homologues biosynthesized in bio-pharmaceutical research. In evaluating, the properties exhibited by some of these compounds [10,11] hypothesized that their quaternary forms possess antifungal, antibacterial and anti-cholestrolic effects in living hosts. Furthermore, the other compounds present in the extract with >1.8% abundance are some rare forms of polyamides and sulfonamides such as N-[2-(dimethylamino)-5-pyrimidinyl] benzene sulfonamide, 9, 12, 15,

Octadecatrien-1-ol and p-Menth-4(8)-en-9-ol see Table 1. The polyamides and sulfonamides are present abundantly in local drug combinations and formulations containing amides and sulfonamides have inhibitory effects on aflatoxigenic molds and antibacterial effects on coliforms [5,12]. However, the other compounds present in trace quantities in the plant extract are butanoic acid ethyl ester, 2-(4-methyl phenyl)-Indolizene and methyl 8, 11, 14-heptadecatrienoate see Table 1. These compounds belong to several classes of organic compound, which include esters, reactive ionic forms and phenols. These compounds are also of interest to pharmacologic research, to attest this, recent studies in Li et al. and Ekanem suggested that these compounds have mild antitumor and antioxidants effects in living hosts [11,13]. Indeed, all these compounds present in the plant *V. amygdalina* have wide range pharmacologic effects underscoring the potency of the plant and justifying the interest it has garnered in recent years among many scientists in Nigeria.

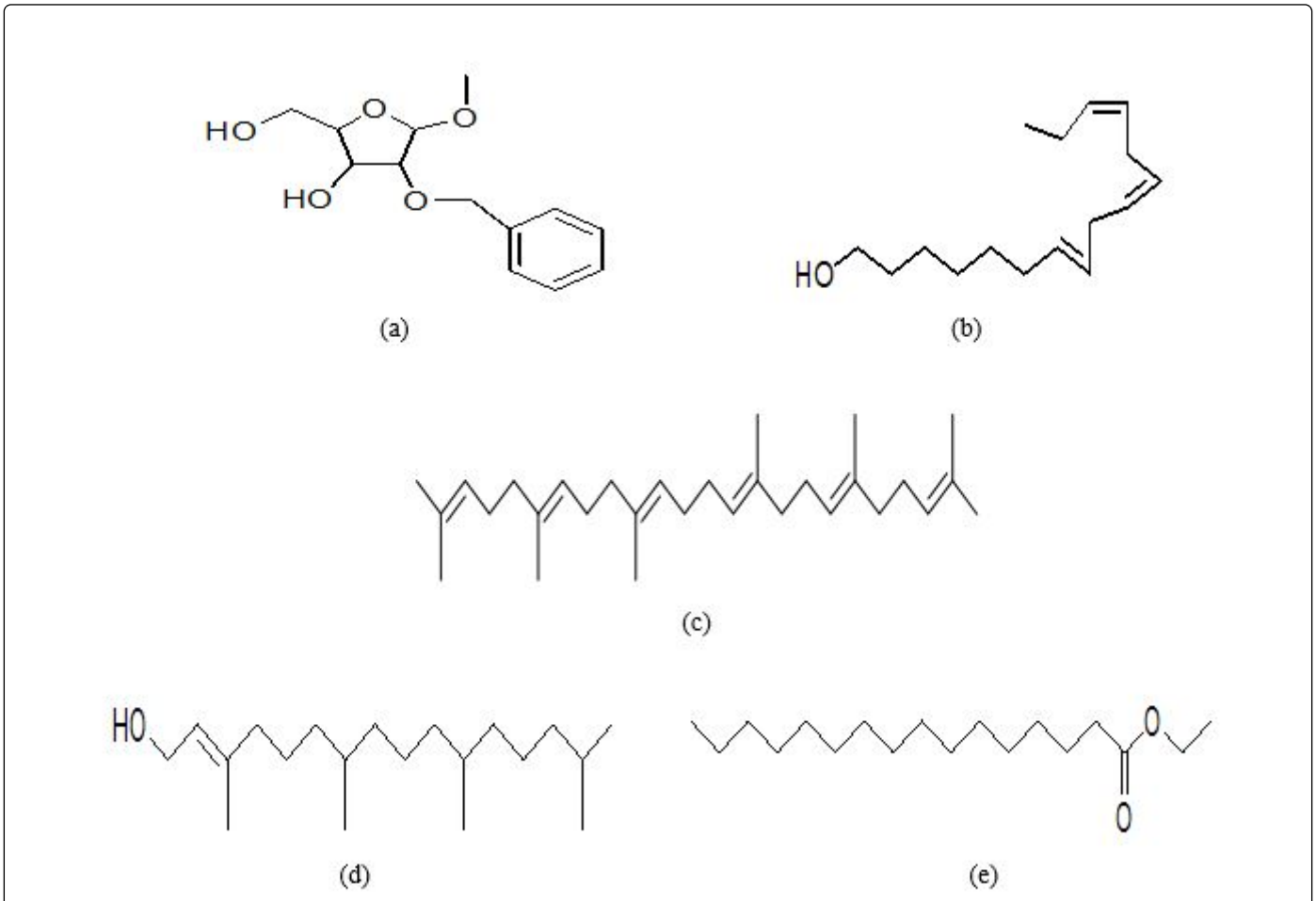

Figure 2: Structures of compounds abundantly present in the plant *V. amygdalina*. Keys: (a)-ethyl-2-O-benzyl-d-arabinofuranoside, (b)-9, 12, 15, octadecatrienoic acid, (c)-squalene, (d)-phytol, (e)-hexadecanoic acid ethyl ester. Authors drew all structures using ChemDraw Ultra 8.0.

Conclusion

This study showed the presence of significant organic compounds in the plant *V. amygdalina* and the quantities available through solvent extraction exhibit that the plant possesses overt pharmacologically active compounds. The study also showed that the plant contains bioactive compounds of interest for further antimicrobial research and provided a study map for further research on the compounds elucidated.

Conflict of Interest

There are no conflicts of interests.

Acknowledgement

The authors will like to acknowledge Mr. Wale Sorungbe of the Department of Biological sciences, Federal University of Technology Akure, Ondo state, Nigeria for the identification of the test plant.

Funding Source

The authors received funding support from the Nigerian Tertiary Education Trust Fund (TETFund) for this research (Grant ID VCPU/TETFund/155).

References

1. Nittya D, Suresh K (2015) A review on ethno-medicinal uses and pharmacology of *Vernonia cinerea* Less. Natural Prod Res 29: 12-22.

2. Faeji C, Oladunmoye M (2017) Comparative evaluation of phytochemical constituents and antioxidant capacity of some ethno-medicinal plants used in Western Nigeria. IOSR J Pharm Biol Sci 12: 5-9.
3. William J, Paul B, Clement Y (2017) Therapeutic mechanisms of *Vernonia amygdalina* delile in the treatment of prostate cancer. Molecules 22: 1594.
4. Elvira E, Kovac-Besovic K, Kalodera Z, Sofic E (2009) Identification and isolation of pharmacologically active triterpenes in betuale cortex, betula pendula roth., Betulaceae. Bosnian J Basic Med Sci 9: 32-38.
5. Igwe K, Madubuike A, Amaku F, Chika I, Otuokere E (2016) GC-MS analysis for structural identification and bioactive compounds in methanolic leaf extract of *Mallotus oppositifolius*. Global J Sci Frontier Res 16: 12-19.
6. Ekanem R (2018) Detection and quantification of potential bioactive compounds in the acetone extract of *Citrullus lanatus* (Melon) seed using GC-MS and FTIR 4 spectrometer. AJOCS 5: 1-5.
7. Huang S, Jin-We L, Zhou W, Hua-Xin P, Jiang-Xu C (2010) Optimization of alkaline extraction of polysaccharides from *Ganoderma lucidum* and their effect on immune function in mice. Molecules 15: 3694-3708.
8. Farid A, Chemat G, Iman H (2012) Plant based natural products extraction: Concept and principles. Int J Mol Sci 13: 21-35.
9. Karishma R, Himanshu D, Usha M (2015) Polyphenols: Methods of extraction. Sci Revs Chem Commun 5: 1-6.
10. Lodhi S, Pawar R, Jain A, Singhai A (2006) Wound healing potential of *Tephrosia purpurea* (Linn.) in rats. J Ethnopharmacol 108: 204-210.
11. Ahmad S, Mohamed T, Zaki M, Rahman A (2011) Extraction of *Jatropha curcas* fruits for antifungal activity against anthracnose (*Colletotrichum gloeosporioides*) of papaya. African J Biotechnol 10: 9796-9799.
12. Cowan M (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12: 564-582.
13. Li A, Zhang Y, Xu R, Chen Y, Li H (2014) Resources and biological activities of natural polyphenols. Nutrients 6: 6020-6047.