Pak. J. Agri. Sci., Vol. 56(x), xxx-xxx; 2019
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/19.7854
http://www.pakjas.com.pk

GENETIC VARIABILITY, ANTIMICROBIAL ACTIVITY AND NATURAL WATER-SOLUBLE VITAMINS CONTENTS OF FIVE ACACIA SPECIES GROWING IN JAZAN REGION, SAUDI ARABIA

Ali I. Sulayli[1], Mahmoud F. Moustafa[1, 2, *] and Ebrahem M. Eid[1, 3]

[1]Biology Department, Faculty of Science, King Khalid University, Abha, Saudi Arabia; [1, 2]Botany Department, Faculty of Science, South Valley University, Qena, Egypt; [1,3] Botany Department, Faculty of Science, Kafr El-Sheikh University Kafr El-Sheikh Egypt
***Corresponding author's e-mail: mfmostfa@kku.edu.sa**

In this study, genetic variability, antimicrobial activity and water-soluble vitamins content were investigated among *Acacia ehrenbergiana*, *Acacia seyal*, *Acacia etbaica*, *Acacia tortilis* and *Acacia asak*. Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR) and mixed primers were used to evaluate the genetic variability among *Acacia* spp. 58 bands resulted from RAPD primers of which 51 unique bands, 7 polymorphic bands and no monomorphic bands with similarity values between 18.37% to 63.38%. ISSR primers showed 75 scorable bands of which 42 unique bands, 31 polymorphic bands and 2 monomorphic bands with similarity values ISSR (25% to 47.06%). Mixed primers showed 71 bands of which 41 unique bands, 29 polymorphic bands and one monomorphic band with similarity values ranged between 29.09% to 46.39%. RAPD and mixed primers separated *Acacia ehrenbergiana* from other investigated species while ISSR primers separated both *Acacia etbaica* and *Acacia tortilis* from other species. Antimicrobial activities were screened against six human pathogenic microorganisms (HPM) and four plant pathogenic fungi (PPF) by using agar well diffusion method. Except extract of dry bark of *Acacia tortilis* and fresh leaves of *Acacia asak* all others had significant antimicrobial activities against HPM and all extracts against PPF showed weak activities. High Performance Liquid Chromatography (HPLC) was used to estimate the vitamin B1, folic acid, B12 and B2 content among *Acacia* spp. The highest content of vitamin B1 and folic acid found in *Acacia seyal* fresh leaves, B12 in *Acacia etbaica* fresh leaves while B2 in *Acacia asak* dry bark. In conclusion, RAPD, ISSR and mixed primers showed ability to distinguish variability among *Acacia* spp., and those plants posses' natural antimicrobials agent and natural vitamins that could be used as an alternative to the synthetic drugs.
Keywords: RAPD, ISSR, mixed, HPM, PPF, HPLC, genetic variability, antimicrobial activity.

INTRODUCTION

Jazan region located in southwestern area of kingdom of Saudi Arabia. Region extended from N 17º 53' 46.30" to N 16º 29' 28.84", E 43º 19' 58.17" to E 41º 35' 36.97" and have altitude ranged up to 2600 meters above the sea level. The area has many plant species about 850 species belong to 98 (Miller and Cope, 1996) of which approximately 141 species distributed in 61 families have been described as medicinal plants. Fabaceae or Leguminosae family is considered as one of the most dominant families in Jazan area, KSA composed mainly from genus *Acacia*. The plants of *Acacia* genus were described as trees or shrubs with branched or unbranched trunks, white or yellow flowers. In traditional medicine, the leaves, stems, flowers, seeds and pods have valuable benefits as antibiotic therapy. Also, *Acacia* spp. have been used as traditional medicine to treat many health diseases such as diarrhea, fever, haemoptysis, leukorrhoea and throat infections (Agrawal and Gupta, 2013). *A. asak* and *A. tortilis* have been reported for healing the gastric ulcer, antiseptic and skin disease (Alfatimi *et al.,* 2007). *A. ehrenbergiana* leaves were evaluated for their anti-inflammatory activity in rats (Mohammad *et al.,* 2017).

Ecologically, *Acacia* species have been reported to play important roles in soil stabilization, industries, and as a source of Arabian gum, fire wood, tannins, fuel, perfumes, paint, ink, proteins and used in cleaning as disinfectant (Isaacs, 1987; Wickens, 1995; Cock, 2011; Seigler, 2003; Arias *et al.,* 2004). A study at northwest of Spain in Europe reported that the expansion of two species of *Acacia* namely *A. dealbata* and *A. melanoxylon* as invasive species due to the plant characterized by high resistance to biotic and abiotic factors. Therefore, became a dominant plant in the forest with annual spread rate (Hernandez *et al.,* 2014).

Investigated the *Acacia* spp. seed oil and fatty acid composition collected from northwest zone of India showed that the lowest oil content (40g kg^{-1}) was in *A. tortilis.* And the seed oil contains linoleic and palmitic, stearic, oleic and arachidic acids which effectively reduced the blood cholesterol (Khan *et al.,* 2012; Embaby and Rayan, 2016). Chemical composition of seeds and pods of *A. ehrenbergiana*, *A. seyal* and *A. tortilis* were investigated in arid and semi-arid

lands of Sudan. The results indicated that there were various content of fibers, crude protein, starch, fat and ash, etc, which conducted high nutritional value (Abdalla *et al.*, 2014; Abdalla *et al.*, 2015; Embaby and Rayan, 2016). The activated carbon that extracted from *A. etbaica* showed effective activity in removing organochlorine pesticides from aquatic bodies (Gebrekidan *et al.*, 2015).

Therefore, in this study five species from *Acacia* genus namely *Acacia ehrenbergiana*, *Acacia seyal*, *Acacia etbaica*, *Acacia tortilis* and *Acacia asak* were selected to investigate the plants therapeutic effects against some human pathogenic microorganisms and plant pathogenic fungi by using agar well diffusion methods. HPLC analysis was applied to reveal natural water-soluble vitamins content among them. This study also aimed to study the relationships and genetic variability and to draw phylogenetic trees by using extracted genomic DNA and numbers of RAPD, ISSR and mixed primers.

MATERIALS AND METHODS

***Plant materials*:** 300 grams from fresh leaves and 100 grams from bark of *Acacia* spp. plants were collected from Jazan region, KSA (Figure 1). The bark parts were dried in shadow for two weeks to ensure the dryness.

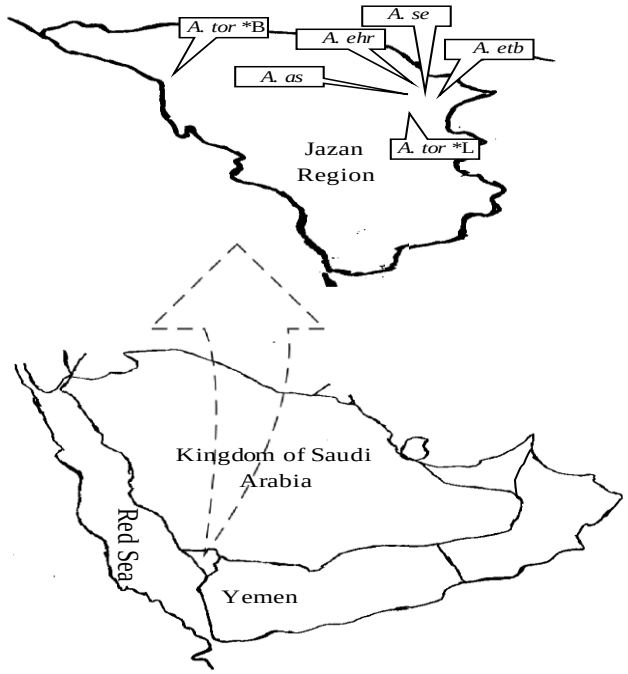

Figure 1. ***Acacia*** **spp. sampling sites in Jazan region, Kingdom of Saudi Arabia.** ***A.ehr*****:** ***Acacia ehrenbergiana, A.se*****:** ***Acacia seyal, A.etb*****:** ***Acacia etbaica, A.tor*** ***L:** ***Acacia tortilis*** **fresh leaves,** ***A.tor*** ***B:** ***Acacia tortilis*** **dry bark,** ***A.as*****:** ***Acacia asak*****.**

***Genomic DNA extraction process and PCR amplification*:** The genomic DNA has been extracted from fresh leaves of each *Acacia* spp. by DNeasy kit provided by QIAGEN-USA COMPANY" and its amount was determined using a Thermo Scientific™ BioMate 3S UV-Visible adjusted at 260 nm. Polymerase chain reaction (PCR) was performed in a final volume of 25 µl composed of 5 µl of genomic DNA template of *Acacia* spp. plants, 12.5 µl of GoTaq DNA polymerase (2.5 mm $MgCl_2$, 2.5 mm of each dNTPs), 5 µl primer and completed using water free nuclease. Seven RAPD, ISSR and mixed primers have been used (Tables 1-3). Amplification procedures was performed in: Initial denaturation at 94°C for 5 minutes followed by 49 cycles of denaturation at 92°C for 1-minute, annealing temperature 29 °C for 1 minute, extension at 72°C for 2 minutes and final extension at 72°C for 7 minutes.

Table 1. RAPD primers for genetic diversity among *Acacai* spp.

RAPD	Sequence of primer (5' – 3')
Oligo 345	5'-GCGTGACCCG-3'
OPM 7	5'-CCGTGACTCA-3'
Oligo 349	5'-GGAGCCCCCT-3'
DO 7	5'-TTGGCACGGG-3'
OPK 8	5'-GAACACTGGG-3'
OPJ 1	5'-CCCGGCATAA-3'
OPE-18	5'-GGACTGCAGA-3'

Table 2. ISSR primers for genetic diversity among *Acacai* spp.

ISSR	Sequence of primer (5' – 3')
Primer 2	5'-GAGAGAGAGAGAGAGAT-3'
UBC 826	5'-ACACACACACACACACC-3'
Primer 1	5'-AGAGAGAGAGAGAGAGC-3'
UBC 888	5'-CACCACACACACACACACA-3'
UBC 823	5'-TCTCTCTCTCTCTCTCC-3'
UBC 840	5'-GAGAGAGAGAGAGAGAGA-3'
Primer 4	5'-CACACACACACACAAG-3'

Table 3. Mixed primers " RAPD + ISSR ":

Mixed	Sequence of primer (5' – 3')
Oligo 345 +Primer 2	5'-GCGTGACCCG-3' + 5'-GAGAGAGAGAGAGAGAT-3'
OPM 7 +UBC 826	5'-CCGTGACTCA-3' + 5'-ACACACACACACACACC-3'
Oligo 349 +Primer 1	5'-GGAGCCCCCT-3' + 5'-AGAGAGAGAGAGAGAGC-3'
DO 7 +UBC 888	5'-TTGGCACGGG-3' + 5'-CACCACACACACACACACA-3'
OPK 8 +UBC 823	5'-GAACACTGGG-3' + 5'-TCTCTCTCTCTCTCTCC-3'
OPJ 1 +UBC 840	5'-CCCGGCATAA-3' + 5'-GAGAGAGAGAGAGAGAGA-3'
OPE-18 +Primer 4	5'-GGACTGCAGA-3' + 5'-CACACACACACACAAG-3'

***Agarose gel electrophoresis*:** 1.47% agarose gel has been used to evaluate the amplified DNA fragments of RAPD, ISSR and mixed-PCR products. Agarose gels have strained with ethidium bromide and run horizontally in 0.5X Tris-borate-EDTA. Electrophoresis buffer covered the gel and run at 70 voltages for 70 minutes. To observe the bands, a gel documentation system (ProXima AQ-4) used as described by (Marsafari and Mehrabi, 2013).

***Data analysis*:** Amplified bands were counted manually as present (1) or absent (0) from the gel to evaluate the similarities among *A. ehrenbergiana, A. seyal, A. etbaica, A. tortilis* and *A. asak*. The matrix of similarity, based on Rogers and Tanimoto's similarity coefficient and squared Euclidean distance was applied to estimate the distances and to depict a dendrogram (Chikkaswamy and Parsad, 2012). The polymorphism percentage was calculated by divided the number of polymorphic bands to the total number of all scorable bands in each primer.

***Antimicrobial activity of Acacia spp.*:** Six human pathogenic microorganisms (HPM) namely *Candida albicans, Klebsiella oxytoca*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*" and four plant pathogenic fungi (PPF) namely, *Alternaria alternata, Mucor racemosus, Penicillium chrysogenum* and *Fusarium oxysporum* were examined by agar well diffusion method (Patel *et al*., 2007; Alamri and Moustafa, 2010; 2012; Alrumman *et al*., 2012; Moustafa *et al*., 2013; Moustafa and Alrumman, 2015).

***Solvent extraction process*:** 300 gm of *Acacia* species fresh leaves added to 700 ml distilled water then crushed and mixed by using a homogenizer mixer. 50 ml from each sample was weighted and divided into 5 tubes each with 10 ml. The dried bark of each sample was crushed into a fine powder by using a homogenizer mixer and 15 grams weighted then divided equally in 5 tubes each with 3 grams. 10 ml from chloroform, alcohol, petroleum ether, methanol and petroleum benzin were added to each plant sample. All samples were placed in rotary shaker at 150 rpm for 48 hours at 25 °C. To evaporate the solvents from each sample, the resultant extract was filtered and kept in incubator at 57 °C till complete evaporation. Each extract was weighed then dissolved in 10 ml of sterile dimethyl sulfoxide (DMSO) and kept at 4 °C for antimicrobial activity test (Moustafa, 2013).

***Antimicrobial activity test*:** As described previously by Alamri and Moustafa, 2010 and 2012; Alrumman *et al*., 2012; Moustafa *et al*., 2013, the antimicrobial activities of solvent extracts gained from *A. ehrenbergiana, A. seyal, A. etbaica, A. tortilis* and *A. asak* extracts were evaluated by using agar well diffusion methods. About 20 ml from previously prepared Mueller-Hinton sterile agar was poured in Petri dishes left to 1 hour for solidifying the media. The media was inoculated using a sterile loop with 0.1 ml from tested microbes. 6 mm hole was made using a sterile cork-borer and then 0.1 ml of the plants extracts added to each hole. All petri dishes were kept at room temperature for 60 minutes to allow the diffusion of the plant extract. Amoxicillin 500 mg was used as positive control while DMSO applied only as a negative control. All dishes were incubated at 29 °C for 48 hours. The sensitivities of tested pathogenic microbe to *A. ehrenbergiana, A. seyal, A. etbaica, A. tortilis* and *A. asak* extracts were determined by measuring the diameter of inhibition zone around the well in millimetres.

***Data analysis*:** Two-ways analysis of variance using of Windows version 10 was applied to examine the significance of data.

***HPLC analysis of water-soluble vitamins*:** High performance liquid chromatography (HPLC) analysis of the fresh leaves and dry bark extracts gained from *A. ehrenbergiana, A. seyal, A. etbaica, A. tortilis* and *A. asak* were performed by using a Shimadzu model HPLC system as described by (Moustafa, 2013).

RESULTS

***Genetic diversity among Acacia spp.*:** 58 bands resulted from applying seven RAPD primers of which 51 unique bands, 7 polymorphic bands and no monomorphic bands. The polymorphism percentage ranged from 0.00% (Oligo 345 and OPE -18) to 25% (OPJ 1) with an average of 11.33% polymorphism (Table 4). The highest genetic similarity value was 63.38% between *A. seyal* and *A. tortilis* also between *A. seyal* and *A. asak* while the lowest similarity value was 18.37% between *A. ehrenbergiana* and *A. etbaica* (Table 5). The dendrogram based on Wards Euclidean methods showed three main clusters (Figure 2).

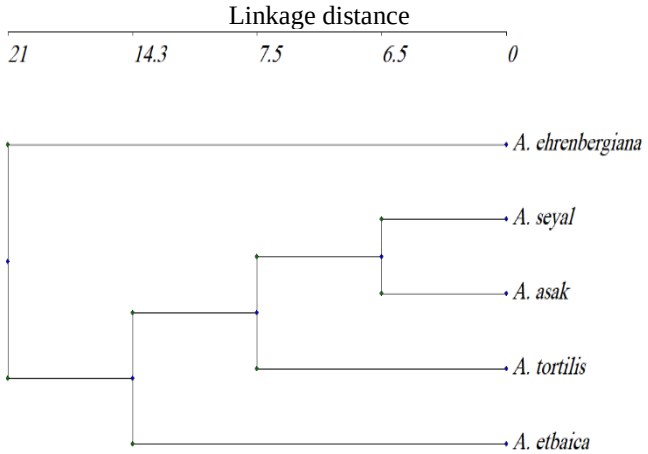

Figure 2. Dendrogram showing the genetic relationship among the five *Acacia* spp. based on RAPD primers data.

First cluster (out-group) included *A. ehrenbergiana* with similarity values ranged from 18.37% to 31.82% with other species. Second cluster included two subclusters, first

Table 4. Polymorphism of seven RAPD primers applied on fresh leaves of five *Acacia* spp.

Primer ID	Total no. of bands	No. of polymorphic bands	No. of monomorphic bands	No. of unique bands	Polymorphism %
Oligo 345	4.00	0.00	0.00	4.00	0.00
OPM 7	9.00	2.00	0.00	7.00	22.22
Oligo 349	15.00	2.00	0.00	13.00	13.33
DO 7	9.00	1.00	0.00	8.00	11.11
OPK 8	13.00	1.00	0.00	12.00	7.69
OPJ 1	4.00	1.00	0.00	3.00	25.00
OPE-18	4.00	0.00	0.00	4.00	0.00
Total	58.00	7.00	0.00	51.00	11.33

Table 5. Genetic similarity among the five *Acacia* spp. based on RAPD primers calculated by Rogers and Tanimoto's coefficient.

Primer ID	*A. ehrenbergiana*	*A. seyal*	*A. etbaica*	*A. tortilis*	*A. asak*
A. ehrenbergiana	100.0				
A. seyal	30.34	100.0			
A. etbaica	18.37	46.84	100.0		
A. tortilis	28.89	63.38	38.10	100.0	
A. asak	31.82	63.38	41.46	56.76	100.0

Table 6. Polymorphism of seven ISSR primers applied on fresh leaves of five *Acacia* spp.

Primer ID	Total no. of bands	No. of polymorphic bands	No. of monomorphic bands	No. of unique bands	Polymorphism %
Primer 2	14.00	6.00	1.00	7.00	42.86
UBC 826	20.00	10.00	00.00	10.00	50.00
Primer 1	00.00	00.00	00.00	0.00	00.00
UBC 888	9.00	5.00	1.00	3.00	55.55
UBC 823	11.00	4.00	00.00	7.00	36.36
UBC 840	5.00	1.00	00.00	4.00	20.00
Primer 4	16.00	5.00	00.00	11.00	31.25
Total	75.00	31.00	2.00	42.00	33.71

Table 7. Genetic similarity among the five *Acacia* spp. based on ISSR primers calculated by Rogers and Tanimoto's coefficient.

Primer ID	*A. ehrenbergiana*	*A. seyal*	*A. etbaica*	*A. tortilis*	*A. asak*
A. ehrenbergiana	100.00				
A. seyal	40.19	100.00			
A. etbaica	42.86	40.19	100.00		
A. tortilis	36.36	25.00	47.06	100.00	
A. asak	38.89	36.36	41.51	30.43	100.00

subcluster included the highest similarity value between *A. seyal* and *A. asak* while second subcluster included *A. tortilis* with similarity values ranged from 28.89% to 63.38% to other species. Third cluster included *A. etbaica* with similarity values ranged from 18.37% to 46.84% with other species.

On the other hand, the seven ISSR primers selected to amplify the genomic DNA of five *Acacia* species produced 75 scorable bands of which 42 unique bands, 31 polymorphic bands and 2 monomorphic bands. The polymorphism percentage ranged from 0.00% (Primer 1) to 55.55% (UBC 888) with an average of 33.71% polymorphism (Table 6). The highest similarity value (47.06%) was between *A. etbaica* and *A. tortilis* while the lowest similarity value (25%) was between *A. seyal* and *A. tortilis* (Table 7). The dendrogram divided the five species into three main clusters (Figure 3). The first cluster included *A. ehrenbergiana* and *A. seyal* with similarity value 40.19%, while the second cluster included *A. asak* with similarity values ranged from 30.43% to 41.51%. The third cluster showed the highest similarity value between *A. etbaica* and *A. tortilis*.

71 bands had been obtained by using seven mixed primers of which 41 unique bands, 29 polymorphic bands and 1

Table 8. Polymorphism of seven mixed primers applied on fresh leaves of five *Acacia* spp.

Mixed Primers ID	Total no. of bands	No. of polymorphic bands	No. of monomorphic bands	No. of unique bands	Polymorphism %
Oligo 345 + Primer 2	17.00	11.00	0.00	6.00	64.70
OPM 7 + UBC 826	14.00	9.00	0.00	5.00	64.28
Oligo 349 + Primer 1	10.00	0.00	0.00	10.00	0.00
DO 7 + UBC 888	11.00	5.00	0.00	6.00	45.45
OPK 8 + UBC 823	0.00	0.00	0.00	0.00	0.00
OPJ 1 + UBC 840	6.00	0.00	0.00	6.00	0.00
OPE-18 + Primer 4	13.00	4.00	1.00	8.00	30.77
Total	71.00	29.00	1.00	41.00	29.31

Table 9. Genetic similarity among the five *Acacia* spp. based on mixed primers calculated by Rogers and Tanimoto's coefficient.

Primer ID	*A. ehrenbergiana*	*A. seyal*	*A. etbaica*	*A. tortilis*	*A. asak*
A. ehrenbergiana	100.0				
A. seyal	35.24	100.0			
A. etbaica	29.09	31.48	100.0		
A. tortilis	30.28	46.39	36.54	100.0	
A. asak	37.86	43.43	39.22	37.86	100.0

monomorphic band. The polymorphism percentage ranged between 0.00% ("Oligo 349 + Primer 1","OPK 8 + UBC 823" and "OPJ 1 + UBC 840") to 64.70% (Oligo 345 + Primer 2) with an average of 29.31% polymorphism (Table 8). The highest similarity value (46.39%) between *A. seyal* and *A. tortilis* while the lowest value (29.09%) between *A. ehrenbergiana* and *A. etbaica* (Table 9).

The dendrogram divided into three main clusters (Figure 4). The first cluster (out-group) included *A. ehrenbergiana* with similarity value ranged from 29.09% to 37.86%, while the second cluster grouped *A. seyal* and *A. tortilis* with the highest value. The third cluster included *A. etbaica* and *A. asak* with similarity value 39.22%.

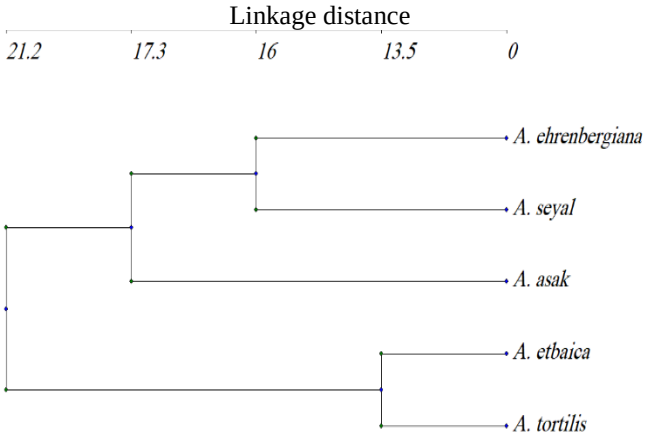

Figure 3. Dendrogram showing the genetic relationship among the five *Acacia* spp. based on ISSR primers data.

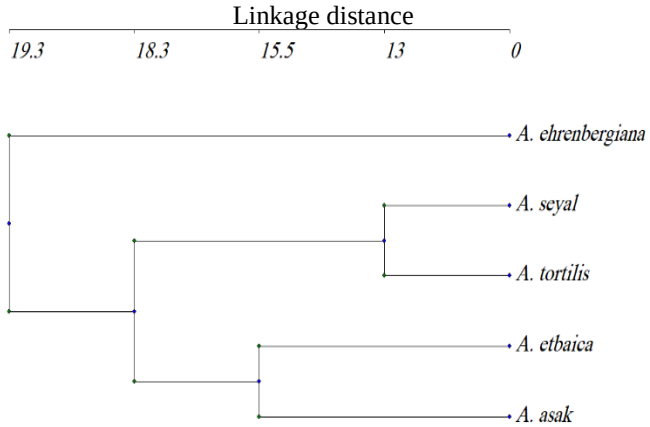

Figure 4. Dendrogram showing the genetic relationship among the five *Acacia* spp. based on mixed primers data.

The genetic diversity based on the sum results of RAPD, ISSR and mixed primers (Table 10). It revealed that the highest similarity value (45.71%) between *A. seyal* and *A. asak* while the lowest similarity value (30.35%) between *A. ehrenbergiana* and *A. etbaica*. The sum data dendrogram divided the five species into three main clusters (Figure 5), the first cluster (out-group) included *A. ehrenbergiana* with similarity values ranged from 30.35% to 36.45% to other species. The second cluster included *A. seyal* and *A. asak* with highest similarity value, while the third cluster grouped *A. etbaica* and *A. tortilis* with similarity value 40.69%.

***Antimicrobial activities of Acacia spp. against HPM and PPF*:** Antimicrobial activities were investigated by using agar well diffusion method against six pathogenic

Table 10. Genetic similarity among the five *Acacia* spp. based on RAPD, ISSR and mixed primers calculated by Rogers and Tanimoto's coefficient.

Primer ID	*A. ehrenbergiana*	*A. seyal*	*A. etbaica*	*A. tortilis*	*A. asak*
A. ehrenbergiana	100.0				
A. seyal	35.55	100.0			
A. etbaica	30.35	38.78	100.0		
A. tortilis	32.04	41.67	40.69	100.0	
A. asak	36.45	45.71	40.69	39.73	100.0

Table 11. Antimicrobial activities of *Acacia* spp. against six HPM.

			Mean diameter of inhibition zone ± standard error					
	Part	Sol.	*C. albicans*	*K. oxytoca*	*S. aureus*	*P. mirabilis*	*K. pneumoniae*	*P. aeruginosa*
P.C.	-	-	1.2±0.00	42.0±1.50	31.0±0.57	36.3±1.80	44.3±2.00	52.6±1.30
A .ehrenbergiana	FL	C	19.0±0.57**	10.3±1.20**	11.0±0.57**	7.3±0.33**	15.3±0.88**	12.3 ±0.60**
		A	18.6±0.33**	11.3±0.67**	11.3±0.67**	7.0±0.58**	15.6±0.33**	13.3±0.33**
		PE	18.0±0.00**	10.0±1.00**	11.0±0.57**	6.6±0.33**	15.3±0.88**	11.6±0.33**
		M	19.6±0.33**	10.6±0.33**	12.0±0.57**	7.0±0.57**	14.6±0.88**	12.6±0.33**
		PB	19.6±0.33**	9.0±0.57**	12.6±0.33**	8.6±0.33**	15.6±0.33**	12.6±0.33**
	DB	C	20.6±0.33**	15.6±0.33**	16.0±0.00**	17.0±1.00**	17.3±0.33**	16.6±0.33**
		A	21.0±0.58**	15.6±0.33**	16.0±0.00**	16.3±0.33**	17.6±0.67**	18.0±0.58**
		PE	20.0±0.57**	16.6±1.20**	17.3±0.33**	16.0±0.57**	18.3±0.88**	17.3±0.66**
		M	21.0±0.57**	16.0±0.57**	15.3±0.33**	16.0±0.57**	17.6±0.33**	17.3±0.33**
		PB	20.3±0.88**	15.6±0.33**	17.6±0.66**	15.6±0.33**	19.6±0.88**	18.3±0.33**
A .seyal	FL	C	25.3±0.67**	19.7±0.88**	18.0±0.58**	17.7±0.88**	21.3±0.88**	19.3±1.20**
		A	25.0±1.73**	18.3±0.33**	19.3±0.88**	19.0±0.58**	24.6±0.33**	19.6±0.67**
		PE	26.0±0.58**	19.0±0.58**	18.6±1.45**	16.6±0.33**	21.3±0.88**	18.6±0.88**
		M	22.3±0.33**	22.3±0.88**	22.3±1.45**	21.3±0.88**	26.0±0.57**	20.0±1.52**
		PB	23.6±1.20**	18.3±0.88**	17.6±0.33**	18.6±0.33**	22.6±0.33**	18.6±0.88**
	DB	C	21.0±0.58**	18.0±0.58**	17.0±0.58**	18.3±0.33**	18.6±1.20**	21.0±0.58**
		A	18.6±0.33**	17.0±1.00**	16.6±0.88**	19.6±1.45**	16.3±0.33**	18.3±0.88**
		PE	21.0±0.58**	16.3±0.33**	16.6±1.20**	17.0±0.58**	18.0±0.58**	19.0±1.53**
		M	20.3±0.33**	17.6±0.33**	17.3±0.88**	19.3±1.20**	16.3±0.88**	19.3±0.33**
		PB	20.0±0.57**	18.6±0.33**	17.3±0.33**	20.0±1.15**	16.0±0.57**	19.3±0.66**
A. etbaica	FL	C	22.6±0.88**	12.0±1.00**	9.3±1.2**	12.3±1.33**	20.0±0.57**	17.3±1.45**
		A	19.6±0.33**	13.3±0.33**	13.0±0.57**	15.3±0.33**	22.3±0.66**	16.3±0.33**
		PE	20.6±0.66**	11.3±0.66**	12.6±2.90**	12.3±0.88**	20.3±0.33**	16.6±0.88**
		M	18.3±0.66**	11.6±0.66**	13.3±0.66**	20.3±1.76**	22.3±0.66**	13.3±0.88**
		PB	19.0±0.57**	14.6±0.33**	11.0±0.57**	16.0±1.00**	21.6±0.66**	14.0±1.52**
	DB	C	21.0±1.00**	11.6±0.33**	13.3±0.66**	12.0±1.15**	13.6±0.33**	13.6±0.33**
		A	20.6±0.66**	11.6±0.88**	12.3±0.33**	11.3±0.33**	15.3±0.66**	13.0±0.00**
		PE	20.0±0.57**	12.6±1.20**	12.3±0.33**	11.6±0.33**	13.3±0.33**	13.3±0.88**
		M	19.6±0.66**	12.3±0.88**	13.3±0.33**	14.3±2.00**	15.6±0.88**	16.0±0.57**
		PB	20.3±0.88**	11.6±0.66**	12.0±0.57**	13.3±0.88**	14.3±0.33**	16.6±0.33**
A .tortilis	FL	C	16.0±0.00**	10.0±0.57**	10.6±0.66**	10.0±0.57**	14.3±0.88**	9.3±0.33**
		A	16.6±0.33**	12.0±1.15**	9.3±0.33**	8.6±0.66**	15.0±0.57**	11.0±0.00**
		PE	15.3±0.33**	10.6±0.66**	9.0±1.00**	8.3±0.88**	14.3±0.33**	8.6±0.33**
		M	16.6±0.67**	13.0±0.58**	11.3±0.33**	9.6±1.30**	15.3±0.33**	11.3±0.33**
		PB	15.6±0.33**	9.3±0.88**	8.6±0.33**	9.6±1.45**	16.0±0.00**	8.6±0.88**
	DB	C	12.3±1.20	7.3±0.33**	7.6±0.66**	12.0±0.00**	11.3±0.33**	7.0±0.00**
		A	10.3±1.76	9.0±0.57**	7.3±0.33**	13.3±0.33**	13.6±0.88**	7.0±0.00**
		PE	11.0±1.52	7.6±0.33**	8.3±0.88**	12.3±0.33**	12.3±0.88**	7.6±0.33**
		M	12.3±0.33	7.6±0.67**	7.0± 0.00**	11.3±0.88**	13.0±0.58**	7.0±0.00**
		PB	12.3±0.33	7.0±0.00**	8.6±0.67**	12.0±0.00**	13.3±0.33**	7.3±0.33**
A. asak	FL	C	8.3±1.33	7.3±0.66**	9.3±0.33**	9.3±0.33**	12.0±1.15**	9.3±0.33**
		A	13.0±2.00	8.3±0.66**	8.6±0.33**	9.6±0.33**	13.0±0.57**	9.3±0.66**
		PE	11.6±0.66	9.0±1.00**	9.3±1.88**	9.6±0.33**	13.0±1.15**	9.3±0.33**
		M	13.0±2.51	10.6±1.20**	9.0±0.57**	10.0±0.57**	13.0±0.57**	8.6±0.66**
		PB	11.0±0.57	10.0±0.57**	9.3±0.33**	10.3±0.88**	15.3±0.88**	10.0±0.57**
	DB	C	20.6±0.88**	16.3±1.20**	17.0±1.73**	19.6±0.88**	18.0±0.57**	18.3±0.88**
		A	18.6±0.33**	14.6±0.33**	17.0±1.15**	16.6±2.90**	17.0±0.00**	17.3±0.88**
		PE	19.6±0.33**	16.3±0.33**	17.0±1.15**	11.6±2.00**	17.3±0.33**	17.0±0.00**
		M	19.6±0.88**	16.3±0.33**	17.6±0.88**	13.6±0.88**	16.6±0.33**	16.6±0.33**
		PB	20.0±0.57**	15.3±0.33**	18.0±0.57**	13.6± 2.40**	16.3±0.88**	17.0±0.00**

P.C.: Positive control: Amoxicillin 500 mg, Negative control: dimethyl sulfoxide, *: p<0.05, **: p<0.01 represent significant difference compared with positive control, FL: Fresh Leaves, DB: Dry Bark, C: Chloroform, A: Alcohol, PE Petroleum Ether, M: Methanol, PB: Petroleum Benzin.

microorganisms; one fungus (*Candida albicans)*, one gram-positive bacteria (*Staphyllococcus aureus)* and four gram-negative bacteria *(Klebsiella oxytoca*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*). And against four plant pathogenic fungi (PPF) that isolated from molded plants parts and fruits namely *Alternaria alternata*,

Mucor racemosus, *Penicillium chrysogenum* and *Fusarium oxysporum*. The mean of recorded inhibition zones against HPM ranged from 6.6±0.33 mm to 26.0±0.58 mm while against PPF in the range between 7.3±0.33 mm to 19.6±0.88 mm. *A. ehrenbergiana*, *A. seyal* and *A. etbaica* fresh leaves and dry bark extracts observed high significant effect against all tested HPM with all solvent used.

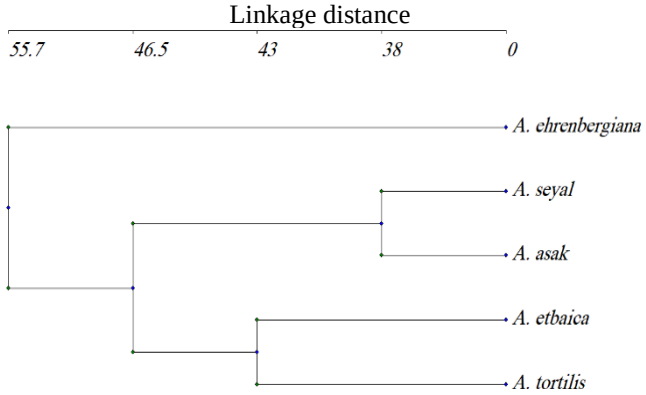

Figure 5. Dendrogram showing the genetic relationship among the five *Acacia* spp. based on all primers data (RAPD, ISSR and mixed primers).

The most susceptible human pathogenic microbes from all studied *Acacia* was *C. albicans* achieving inhibition zone between (16.6±0.33 mm to 26±0.58 mm), followed by methanol and chloroform extract of *A. seyal* fresh leaves against *K. oxytoca*, *S. aureus*, *K. pneumonia and P. aeruginosa* in the range between (21±0.58 mm to 26±0.57 mm). The lowest inhibition zone (6.6±0.33 mm) observed from *A. ehrenbergiana* fresh leaves petroleum ether against *P. mirabilis* as shown in Table 11. The most susceptible plant pathogenic microbe was *A. alternata* whereas the inhibition activity of chloroform extract of *A. ehrenbergiana*, and from dry bark petroleum benzin extract of *A. seyal* and from dry bark chloroform of *A. etbaica* and from chloroform extract of *A. tortilis* and dry bark methanol of *A. asak* in the range between 16.3±2.03 mm to 19.6±0.88 mm. The lowest inhibition zones towards various PPF achieved from leaves chloroform extract, methanol extract, petroleum ether and petroleum benzin extracts of *A. ehrenbergiana*, *A. seyal* and *A. etbaica* recording inhibition zone between 7.3±0.33 mm to 8.6±0.88 mm as shown in Table 12.

***Quantification of some water-soluble vitamins in five Acacia spp.*:** Results obtained from fresh leaves and dry bark extracts showed variations in the amount of vitamin B1, folic acid, B12 and in B2 among the five *Acacia* spp. The highest amount of vitamin B1 observed from fresh leaves of *A. seyal* (343.98 µg/ml), followed by *A. etbaica* fresh leaves (139.08 µg/ml), *A. tortilis* fresh leaves (35.35 µg/ml), *A. asak* fresh leaves (4.7 µg/ml), *A. asak* dry bark (1.4 µg/ml) as shown in (Figure 6). The maximum content of folic acid obtained from *A. etbaica* and *A. seyal* fresh leaves (696.65 µg/ml, 905.46 µg/ml, respectively), followed by *A. ehrenbergiana* dry bark (237.75 µg/ml) while the lowest content obtained from *A. tortilis* dry bark (0.342 µg/ml). Variation in the content of folic acid were found also in the *A. ehrenbergiana* fresh leaves (87.05 µg/ml), in *A. asak* fresh leaves (75.56 µg/ml), in *A. seyal* dry bark (65.97 µg/ml), in *A. asak* dry bark (30.25 µg/ml), in *A. tortilis* fresh leaves (13.98 µg/ml) and in *A.*

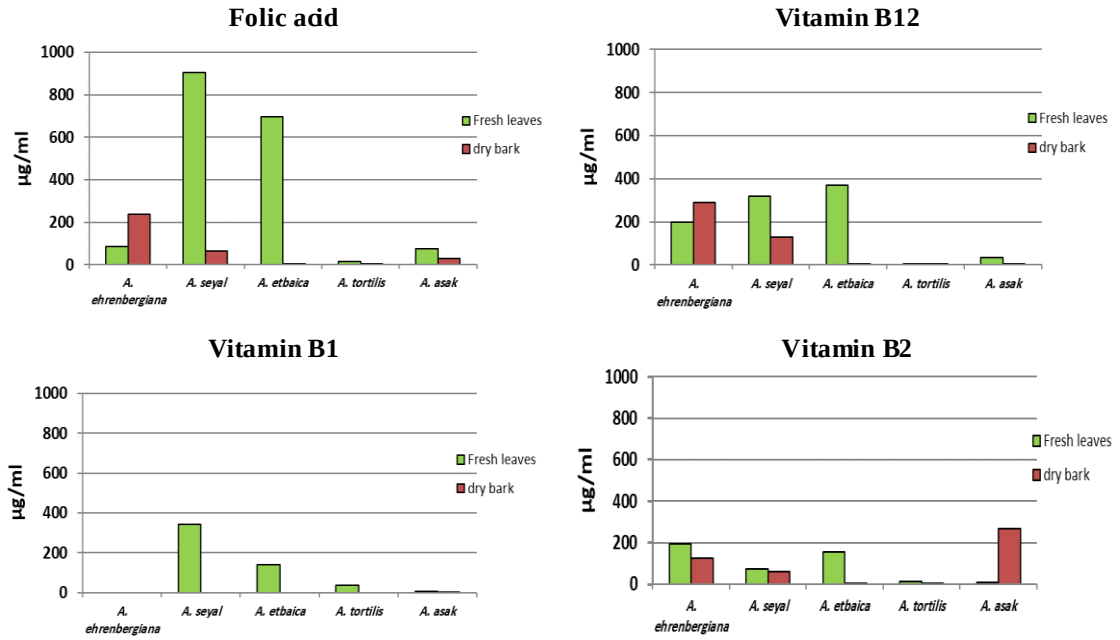

Figure 6. Concentration of soluble water vitamins in five *Acacia* spp.

Table 12. Antimicrobial activities of *Acacia* spp. against four PPF.

			Mean diameter of inhibition zone ± standard error			
	Part	Sol.	*A. alternata*	*M. racemosus*	*P. chrysogenum*	*F. oxysporum*
P.C.	-	-	14.6±0.50	9.6±0.88	10.3±0.66	12.3±0.88
A. ehrenbergiana	FL	C	17.6±0.33	9.6±0.33	8.6±0.88	11.6±1.20
		A	14.6±1.45	9.6±0.88	9.3±1.20	10.0±0.57
		PE	16.3±0.88	10.6±0.33	9.0±0.57	11.0±0.57
		M	15.6±1.30	11.0±0.57	9.3±1.20	9.6±0.33
		PB	12.6±0.66	12.0±0.57	9.0±0.57	9.0±1.15*
	DB	C	14.3±2.60	13.6±0.66**	10.3±0.33	11.6±0.88
		A	12.3±0.88	13.3±0.66**	10.6±0.66	11.3±0.66
		PE	15.3±2.18	11.6±0.66	9.6±0.33	9.0±0.57*
		M	16.6±1.76	13.0±1.00**	8.6±1.20	11.3±0.33
		PB	16.0±0.57	12.6±0.33*	10.6±0.33	10.0±1.52
A. seyal	FL	C	17.6±2.72	10.0±0.57	7.3±0.33	9.0±0.00*
		A	11.6±2.90	10.3±1.66	9.6±0.33	8.6±0.33*
		PE	10.0±0.00	9.6±1.45	10.3±1.20	8.3±0.33*
		M	13.6±3.52	7.3±0.33	9.3±1.20	8.6±0.88*
		PB	11.3±2.30	9.0±1.00	10.0±0.57	8.0±0.57**
	DB	C	18.3±1.76	13.3±0.33	10.6±0.33	10.0±1.57
		A	14.0±2.08	13.0±1.15	11.6±1.45	10.3±0.88
		PE	14.3±1.20	14.3±2.02*	11.0±1.00	9.3±1.85
		M	10.3±3.30	10.0±0.57	11.3±0.33	8.3± 0.88*
		PB	19.6±0.88	11.0±0.57	11.3±0.66	8.6± 0.88*
A. etbaica	FL	C	12.3±1.86	9.6±1.67	9.3±0.88	10.0±0.58
		A	10.0±2.51	10.0±1.00	9.0±0.57	9.3±1.30
		PE	8.0±0.57	9.3±0.66	8.6±0.33	10.3±0.88
		M	12.3±2.30	11.0±1.53	8.3±0.67	12.0±1.53
		PB	12.6±2.90	10.0±0.57	8.6±0.33	8.3±0.33*
	DB	C	16.3±2.03	10.6±0.88	10.0±0.58	10.3±1.20
		A	15.3±3.84	10.6±0.66	9.6±0.33	10.0±0.57
		PE	16.0±4.50	12.3±0.33	11.3±0.33	10.3±0.33
		M	9.0±1.00	11.6±0.67	10.0±0.58	10.0±0.58
		PB	9.3±1.45	12.3±1.20	9.0±1.15	10.0±1.15
A.tortilis	FL	C	16.0±2.08	12.6±0.33	11.6±0.66	11.6±0.33
		A	14.3±2.30	14.0±0.57	11.6±0.33	11.0±1.15
		PE	15.0±1.00	13.3±0.33	11.3±0.66	11.3±0.33
		M	15.0±1.00	12.3±0.33	12.3±1.45	12.3±0.33
		PB	13.3±0.66	13.0±1.00	12.3±0.33	12.0±1.00
	DB	C	12.3±1.45	11.0±1.15	12.0±1.00	10.3±0.33
		A	12.6±0.33	13.3±1.20	10.6±0.33	11.0±1.15
		PE	13.6±0.88	10.3±2.84	12.3±0.33	10.3±0.33
		M	11.6±0.33	11.0±0.57	13.3±0.88*	11.0±0.57
		PB	11.0±1.73	11.3±2.02	9.6±0.88	11.3±0.33
A. asak	FL	C	13.0±1.52	14.3±1.45*	13.3±0.88*	12.3±0.66
		A	12.0±0.00	15.6±1.45**	12.0±0.57	11.6±0.33
		PE	12.0±1.00	15.0±1.52*	10.6±0.33	11.6±0.33
		M	11.6±0.66	14.3±1.85*	13.0±0.57	12.3±1.20
		PB	11.6±0.66	13.3±0.33	12.6±0.88	13.3±0.33
	DB	C	15.3±2.60	15.0±0.57*	11.0±1.00	11.0±0.57
		A	16.3±2.02	12.0±0.57	10.0±1.00	11.6±0.33
		PE	14.3±1.20	15.3±0.88**	10.0±0.57	11.0±1.73
		M	17.0±1.52	13.0±1.15	10.6±0.33	9.0±0.00
		PB	12.0±0.00	13.3±1.20	8.0±1.00	9.6±1.45

P.C.: Positive control: Amoxicillin 500 mg, Negative control: dimethyl sulfoxide, *: p<0.05, **: p<0.01 represent significant difference compared with positive control, FL: Fresh Leaves, DB: Dry Bark, C: Chloroform, A: Alcohol, PE Petroleum Ether, M: Methanol, PB: Petroleum Benzin.

etbaica dry bark (1.58 µg/ml) as shown in (Figure 6). Vitamin B12 found to be very high in *A. etbaica* fresh leaves (370.45 µg/ml) followed by *A. seyal* fresh leaves (320.5 µg/ml), *A. ehrenbergiana* dry bark (289.4 µg/ml), *A. ehrenbergiana* fresh leaves (198.47 µg/ml) and *A. seyal* dry bark (127.48 µg/ml). The lowest amount found in *A. asak* fresh leaves (34.38 µg/ml), *A. tortilis* fresh leaves (4.37 µg/ml), *A. etbaica* dry bark (2.98 µg/ml), *A. asak* dry bark (1.64 µg/ml) and in *A. tortilis* dry bark (1.3 µg/ml) as shown in (Figure 6). With regards to vitamin B2, the maximum content obtained from *A. asak* dry bark (267.46 µg/ml) followed by *A. ehrenbergiana* fresh leaves (194.19 µg/ml) and *A. etbaica*

fresh leaves (155.74 µg/ml). The moderate amount was found in *A. ehrenbergiana* dry bark (123.07 µg/ml), *A. seyal* fresh leaves (71.58 µg/ml) and in dry bark (62.37 µg/ml). While the lowest content found in *A. tortilis* fresh leaves (14.52 µg/ml), *A. asak* fresh leaves (8.97 µg/ml), *A. etbaica* dry bark (1.02 µg/ml) and in *A. tortilis* dry bark (0.66 µg/ml) as shown in (Figure 6).

DISCUSSION

***Genetic Variability*:** In this study either RAPD, ISSR and mixed primers showed effective identification for all investigated *Acacia* species. Seven RAPD primers yielded fifty-eight bands ranged between four bands to fifteen bands for the investigated primers. The polymorphic bands found to be less than unique bands. High unique bands percentage among studied plants indicated there were differences in the genomic content while the presence of polymorphic and monomorphic bands indicated also there were similarities among them. Similar results were found by other researchers for example Moustafa *et al.,* (2016), found higher unique bands in *Ziziphus spina-christi* L. plant, by using twelve RAPD primers on revealing the genetic diversity, however polymorphism percentage were not in accordance with this study, this may be due to that RAPD primers have been applied to same species population not to different species. However, other studies showed that there was a high polymorphism rate than these results, for example, Ramirez *et al.,* (2014) reported that RAPD primers applied on 24 individuals of *Dioscorea* spp. yielded 302 bands with polymorphism percentage with an average of 58.27%. Mei *et al.,* (2017), also reported that 185 bands obtained from 20 RAPD primers applied on 9 samples of *Penthorum Chinese* plants whereas the polymorphic bands represented 68.6% with an average of 9.25 band/primer. Ntuli *et al.,* (2015) studied the genetic variation among 7 samples of *Cucurbita pepo* plants by using 9 RAPD markers yielded 100 bands with an average of 11.11%. Thilaga *et al.,* (2017) screened the genotypes of *Betousa stylophora* Swinhoe by using 5 RAPD primers which yielded 32 bands and explained the differences in polymorphism due to the study have been applied on the same species in different condition while our study was on five species of the same genus. This research also showed that ISSR primers generated seventy-five bands ranged from five bands (UBC 840) to twenty bands (UBC 826). Unique bands represented by forty-two bands (56%) with an average of 6 bands/primer that support the results obtained by the investigated RAPD primers. Unique bands found to be the highest among the recorded other bands value and this again ensure the differences in DNA fingerprints of the five *Acacia* spp. and proof that each species have its own genetic structure. Monomorphic bands represented by two bands (2.67%) with an average of 0.28 band/primer. While polymorphic bands represented by thirty-one bands (41.33%) with an average of 4.43 bands/primer. The polymorphism percentage obtained by six primers represent 39.33% which higher than that observed by RAPD primers (15.87%). Almost the same polymorphism rate has been reported from other previous studies by many researchers, for example, Alturki and Basahi (2015) reported that the polymorphism percentage were in between 40% to 100% by using 14 ISSR primers on 27 landraces of Hassawi rice. Ramirez *et al.,* (2014) showed that the polymorphism rate observed by using ISSR primers (81.93%) of *Dioscorea* spp. was higher than that observed from RAPD primers (58.27). Moustafa *et al.,* (2016) reported also higher polymorphism percentage obtained from ISSR primers (61.49%) than RAPD primers (40.11%) applied on five samples of *Z. spina*. The result of this study gave low polymorphic bands percentage with all primers used which supported by many other studies. Vanijajiva (2012) investigated the genetic diversity of 14 cultivars of *Durio zibethinus* Murr revealed by using 5 ISSR primers found that 50 bands, of which, 19 bands were polymorphic with an average between 30% to 42%. While higher polymorphic bands were given by Chen *et al.,* (2014) who studied the genetic variation of 10 populations of *Magnolia wufengensis* by using 10 ISSR primers and the polymorphic band represented by 87.7% of total bands obtained. Ganopoulos *et al.,* (2015) also reported that by using of 6 ISSR primers to screen the genetic diversity of 22 samples of *Opuntia ficus-indica* found 57 bands of which 50.21% polymorphic bands.

Mixed primers (RAPD+ISSR) revealed the genetic relationships among *Acacia* spp. by obtaining seventy-one bands that ranged between six bands to seventeen bands. Forty-one bands observed as unique bands with an average of 5.86 bands/primer. Monomorphic bands represented by only one band with an average of 0.14 band/primer. While, the polymorphic bands represented by twenty-nine bands with an average of 4.14 bands/primer. Unique bands found to be the highest value of bands same as RAPD and ISSR primers results. Again, this support that these plants belongs to the same genus and clearly indicating that each plant has specific DNA fingerprint by using such types of primers. Mixed primers gave the highest percentage of polymorphism (51.3%) than ISSR primers (39.33%) and RAPD primers (15.87%). This may be due to that there was difference in annealing site to which each primer either alone or mixed that could adhere with specific site to the genome of *Acacia* spp. Also, there are many factors affecting the genetic diversity among *Acacia* spp. grown in Saudi, for example, plant locations, environmental condition, plant adaption to the specific environmental condition, and many other biotic and abiotic factors. In agreement with previous research that showed the genetic composition of *Qureques serrata* did not differ among population, but it differ between populations growing on various topography in the Chichibu Mountains of China country (Ohsawa *et al.,* 2008). And this was interpreted

that the variation between populations of the same species were influenced by many factors such as geographical factors, horizontal distribution, large population size and the gene flow potential. The results of polymorphisms rate found in this study have been well characterized in other studies in investigating different species belongs to the same genus, whereas three *Euphorbia* species tested by RAPD, ISSR markers and GC-MS techniques. It was found that polymorphisms rate ranged between (44.37%- 29.14%) for RAPD, (46.75%-37.87%) for ISSR and (44.69%-36.88) for sum of all data (Moustafa *et al.,* (2016). In conclusion, biosystematics tools including RAPD, ISSR and mixed markers could be applied to study the relatedness among *Acacia* spp.

***Antimicrobial properties and water-soluble vitamins of five Acacia spp.*:** At present time, microbial resistance to antibiotics has a major issue against development of new synthetic drug in addition the synthetic drugs have many side effects and high-cost price. Also, plants extract showed significant antimicrobial activities against a lot of microbial pathogens which cause diseases to human, animals, crops and many other organisms (Hashem *et al.,* 2015). Therefore, many scientists search for alternative therapeutic agents with no side effects, low-cost price and safe usage originated from the plant. This study screened antimicrobial activities of five *Acacia* spp. against six human pathogenic microorganisms (HPM) and against four plant pathogenic fungi (PPF).

The results showed that there were high antimicrobial activities obtained from fresh leaves and dry bark extracts of *A. ehrenbergiana*, *A. seyal* and *A. etbaica* against all tested HPM and little activity against PPF. It was found there were variation in the inhibition activity for each the plant part and types of solvent and types of tested microbes. The zones of inhibition against HPM ranged from 7 to 28 mm as for example the highest inhibition zone observed from alcohol extract of fresh leaves of *A. seyal* against *Candida albicans*. This support previous study that either the Acacia spp. namely *Acacia karroo* and *Acacia nilotica* parts or their excaudate had potent antimicrobial activities against *S. aureus* and *K. pneumonia (*Alawi *et al.,* 2018; Ali *et al.,* 2017). The obtained results clearly reveled that every species has its own antimicrobial activities. For example, the results obtained from *A. tortilis* and *A. asak* various extracts showed high antimicrobial effect against all tested HPM except *C. albicans*. *A. tortilis* extracts gained from fresh leaves extracts showed significant antimicrobial effect against *C. albicans* observed while dry bark found to be possessed low inhibition effect. *A. asak* showed significant antimicrobial activity against *C. albicans* observed with dry bark extracts while fresh leaves extracts showed low activity. These differences in antimicrobial activities between species supported by previous studies. For example, Alfatimi *et al.,* (2007) found that two *Acacia* spp. namely, *A. asak* and *A. tortilis* differed from each other in their antimicrobial activities against *S. aureus, P. aeruginosa* and *C. albicans*. Stem bark petroleum ether and ethyl acetate extracts of *Acacia nilotica* showed moderate antimicrobial activity against *Bacillus polymyxa*, *B. subtilis*, *B. megaterium*, *Vibrio cholera*, *Salmonella typhi*, *Shigella flexneri* and *P. aeruginosa* while methanol extract showed no inhibition activity (Ali *et al.,* 2017). Also, Alfatimi *et al.,* (2007) reported moderate antimicrobial activity obtained from methanol extracts of *A. asak* stem cortex, *A. nilotica* leaves and *A. tortilis* fruit against *S. aureus*, *B. subtilis* and *Micrococcus flavus*. In other study it was found that ethanol extracts gained from *A. tortilis* aerial parts had potent antimicrobial effect against *S. aureus, P. aeruginosa* and *C. albicans* (Alajmi *et al.,* 2017). Therefore, those selected pathogenic microbes that showed resistance to many synthetic antibiotics to be susceptible to the natural solvent extracts of investigated *Acacia* spp. is challenging to be used as alternative natural drugs. Selected human pathogenic microbes cause many diseases to the human for example, *C. albicans* which responsible for nosocomial bloodstream infection (Wisplinghoff *et al.,* 2013) and *P. aeruginosa* normally infected human lungs (Solano *et al.,* 2008).

With regards to the plant pathogenic microbes, there was no significant effects against *A. alternata* while the only significant effect was found against *P. chrysogenum* of dry bark methanol extract of *A. tortilis* and fresh leaves chloroform extract of *A. asak*. In agreement of previous study that there was no activity against *A. alternata* of *Argemone ochroleuca* Sweet latex other than investigated pathogenic plants (Moustafa, *et al.,* 2013). Variations among type of tested plant, solvent and specific microbes against PPF were also observed, for example, *A. ehrenbergiana* showed significant activities against *M. racemosus* only from solvents of dry bark extracts except petroleum ether. While against *F. oxysporum* fresh leaves petroleum benzin and dry bark petroleum ether extracts were the only solvents gave significant activities. *A. seyal* fresh leaves extracts and dry bark (methanol and petroleum benzin) extracts showed significant activities against *F. oxysporum* while against *M. racemosus* the only significant activity observed from dry bark petroleum ether extract. This type of inhibition activity also supported by Alfatimi *et al.,* (2007), who reported that the antimicrobial activity of methanol extract gained from *A. asak*, *A. nilotica* and *A. tortilis* had varied inhibition activities against *Candida krusei*, *Aspergillus fumigatus*, *Absidia corymbifera* and *Trichophyton mentagrophytes*. Also, Ali *et al.,* (2017) reported weak activity of petroleum ether extract of *A. nilotica* against *Candida arrizae*, *A. fumigatus* and *A. niger* while methanol extract had no activity.

Since obtaining natural vitamins from plants is a safer choice as it has no side effect and it considered to be hazardous when taken beyond their recommended dosage in synthetic form. In addition, either fruits or leaves in some Arabian localities taken directly in case of health problem. Natural plant vitamin namely B1, B2 and B12 reported t have a lower risk to cause

premenstrual syndrome (PMS) (Bedoya *et al.,* 2011). If vitamins B1 and B2 obtained from plant sources like wheat it would stimulate 24 loci in genomic DNA that responsible of synthesis of vitamin B1 and B2 (Li *et al.,* 2017). Therefore, by using HPLC some soluble vitamin in the five Acacia plant have been analyzed. It was found that there was great variation in the amount of investigated vitamins among tested *Acacia* spp. indicating that these vitamins in addition beneficial biological properties also could be used to characterize and identify specific plant species.

***Conclusion*:** RAPD, ISSR and mixed primers could be effective identification method to investigate the genetic variability among *Acacia* spp. Antimicrobial activity gained from tested *Acacia* against HPM a challenge to use those plants as an alternative pharmaceutical drug whereas less antifungal effect against PPF need more lab works either to isolate the specific antifungal compound or to proof the chemicals present in the plants have fewer antifungal properties. Variable concentrations of natural vitamins found in *Acacia* spp. could be useful to livestock/animals that require the isolation and also support the variability among different plant species in their chemicals content.

***Acknowledgments*:** Authors would like to thank King Abdul Aziz City for Science and Technology (KACST) for the financial support of this work under the grant number: 1-17-01-010-0016.

REFERENCES

Abdalla, M., I. Babiker and K. Elkalifa. 2014. Potential nutrient composition of A*cacia seyal* fruits as fodder for livestock in dry lands of Sudan. Dev. A. Chem. 1:25-30.

Abdalla, M., I. Babiker and K. Elkalifa.2015. Chemical analysis of *Acacia ehrenbergiana* (Salam) tree fruits (seeds and pods) as dry season supplement for livestock in arid and semi-arid lands of Sudan. Animal Rev. 2:76-80.

Agrawal, N. and U. Gupta. 2013. Evaluation of hypoglycemic and antihyperglycemic effects of *Acacia tortilis* seed extract in normal and diabetic rats. Int. J. Pharm. Tech. Res. 5:330-336.

Alajmi, M., P. Alam, S. Alqasoumi, N. Siddiqui, O. Basudan, A. Hussain, F. Husain. and A. Khan. 2017. Comparative anticancer and antimicrobial activity of aerial parts of *Acacia salicina*, *Acacia laeta*, *Acacia hamulosa* and *Acacia tortilis* grown in Saudi Arabia. Saudi Pharm. J. 25:1248–1252.

Alamri, S. and M. Moustafa. 2010. Antibacterial activity of the latex of *Argemone ochroleuca* Sweet. Saudi Med. J. 31:1207-1210.

Alamri, S. and M. Moustafa. 2012. Antimicrobial property of three medicinal plants from Saudi Arabia against some clinical isolates of bacteria. Saudi Med. J. 33:272-277.

Alawi, S., M. Hossain and A. Abusham. 2018. Antimicrobial and cytotoxic comparative study of different extracts of Omani and Sudanese gum *Acacia*. Beni-Suef University j. basic appl. Sci. 7:22-26.

Alfatimi, M., M. Wurster, G. Schroder and U. Lindequist. 2007. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J. Eth. Pharm. 111:657-666.

Ali, M., S. Haque, M. Kabir, S. Rana and M. Haque. 2017. A comparative study of in vitro antimicrobial, antioxidant and cytotoxic activity of *Albizia lebbeck* and *Acacia nilotica* stem bark. Bulletin of Faculty of Pharmacy, Cairo University. 56:34-38.

Alrumman, S., M. Moustafa and S. Alamri. 2012. Anti-bacterial and anti-fungal investigation of *Astragalus atropilosulus* subsp. *abyssinicus*. Afri. J. Mic. Res. 6:6365-6369.

Alturki, T. and M. Basahi. 2015. Assessment of ISSR based molecular genetic diversity of Hassawi rice in Saudi Arabia. Saudi J. Bio Sci. 22:591-599.

Arias, M., J. Gomez, N. Cudmani, M. Vattuone and M. Isla. 2004. Antibacterial activity of ethanolic and aqueous extracts of *Acacia aroma* Gill. Ex Hook et Arn. Life Sci. 75:191-202.

Bedoya, C., J. Manson, S. Hankinson, W. Willett, S. Johnson, L. Taber, A. Ronnenberg, C. Bigelow and E. Johnson. 2011. Dietary B vitamin intake and incident premenstrual syndrome. Am. J. Clin. Nut. 93:1080-1086.

Chen, L., F. Chen, S. He and L. Ma. 2014. High genetic diversity and small genetic variation among populations of *Magnolia wufengensis* (Magnoliaceae), revealed by ISSR and SRAP markers. Ele. J. Bio. 17:268-274.

Chikkaswamy, B. and M. Prasad. 2012. Evaluation of genetic diversity and relationships in mulberry varieties using RAPD and ISSR molecular markers. Int. J. Mol. Bio. 3:62-70.

Cock, I. 2011. Medicinal and aromatic plants Australia, in Ethnopharmacology section, Biological, Physiological and Health Sciences, Encyclopedia of Life Support Systems (EOLSS), Developed under the Auspices of the UNESCO, EOLSS Publishers, Oxford, UK.

Embaby, H. and A. Rayan. 2016. Chemical composition and nutritional evaluation of the seeds of *Acacia tortilis* (Forssk.) Hayne spp. raddiana. Food Chem. 200:62-68.

Ganopoulos, I., A. Kalivas, N. Kavroulakis, A. Xanthopoulou, A. Mastrogianni, G. Koubouris and P. Madesis. 2015. Genetic diversity of Barbary fig (*Opuntia ficus-indica*) collection in Greece with ISSR molecular markers. Plant Gene. 2:29-33.

Gebrekidan, A., M. Teferi, T. Asmelash, K. Gebrehiwet, A. Hadera, K. Amare, J. Deckers and B. Bruggen. 2015. *Acacia etbaica* as a potential low-cost adsorbent for removal of organochlorine pesticides from water. J. Water Res. Prot. 7:278-291.

Hashem, M., S. Alamri, A. Shathan, S. Alrumman and M. Moustafa. 2015. Antibacterial Behavior of Weed Plants from

Aseer, Saudi Arabia and Their Possible Mode of Action. Int. J. Sci. Res. 4:2491-2499.

Hernandez, L., J. Fernandez, I. Canellas and A. Cueva. 2014. Assessing spatio-temporal rates, patterns and determinants of biological invasions in forest ecosystems. The case of *Acacia* species in NW Spain. Forest Ecology and Management. 239:206-213.

Isaacs, J. 1987. Bush Food: Aboriginal food and herbal medicine. Sydney: Lansdowne Publishing Pty Ltd. Pp.256.

Khan, R., R. Srivastava, M. Khan, P. Alam, M. Abdin and Mahmooduzzafar, 2012. Variation in oil content and fatty acid composition of the seed oil of *Acacia* species collected from the northwest zone of India. J. Sci. Food Agri. 92:2310-2315.

Li, J., J. Liu, W. Wen, P. Zhang, Y. Wan, X. Xia, Y. Zhang and Z. He. 2017. Genome-wide association mapping of vitamins B1 and B2 in common wheat. Crop. J. 6:263-270.

Marsafari, M. and A. Mehrabi. 2013. Molecular identification and genetic diversity of Iranian date palm (*Phoenix dactylifera L*.) cultivars using ISSR and RAPD markers. Aust. J. Crop Sci. 7:1160-1166.

Mei, Z., X. Zhang, M. Khan, S. Imani, X. Liu, H. Zou, C. Wei and J. Fu. 2017. Genetic analysis of *Penthorum chinense* Pursh by improved RAPD and ISSR in China. Elec. J. Bio. 30:6-11.

Miller, A. and T. Cope. 1996. Flora of the Arabian Peninsula & Socotra. Edinburgh University Press, Edinburgh. PP. 1-586.

Mohammad, I., M. Alsafi, M. Almahdi, T. Elsammani and M. Mudawi. 2017. Evaluation of anti-inflammatory activity of *Acacia ehrenbergiana* (Salam) leaves by in-vivo and in-vitro models. Pharm. Sci. 4:26-32.

Moustafa, M. and S. Alrumman. 2015. First report about pharmaceutical properties and phytochemical analysis of *Rosa abyssinica* R. Br. ex Lindl. (Rosaceae)". Pak. J. Pharm. Sci. 28:2009-2017.

Moustafa, M., S. Alamri, H. Taha and S. Alrumman. 2013. In vitro antifungal activity of *Argemone ochroleuca* Sweet latex against some pathogenic fungi. Afri. J. Bio. 12:1132-1137.

Moustafa, M., S. Alrumman and A. Hesham. 2016. Biological activities of some *Acacia* spp. (Fabaceae) against new clinical isolates identified by ribosomal RNA gene-based phylogenetic analysis. Pak. J. Pharm. Sci. 29:221-229.

Moustafa, M., A. Hesham, M. Quraishi and S. Alrumman. 2016. Variation in genetic and chemical constituents of *Ziziphus spina-cristi* L. populations grown at various altitudinal zonation up to 2227 m height. J. Gen. Eng. Bio. 14:349-362.

Moustafa, M., O. Mostafa, D. Alshahrani and S. Alrumman. 2016. An application of genetics-chemicals constituents to the relatedness of three *Euphorbia* species. Biologia. 71:1240-1249.

Ntuli, N., P. Tongoona and A. Zobolo. 2015. Genetic diversity in *Cucurbita pepo* landraces revealed by RAPD and SSR markers. Sci. Hort. 189:192-200.

Ohsawa, T., Y. Saito, H. Sawada and Y. Ide. 2008. Impact of altitude and topography on the genetic diversity of *Quercus serrata* populations in the Chichibu Mountains, central Japan. Flora - Morphology, Distribution, Fun. Eco. Plants. 203:187-196.

Patel, S., V. Nithin and S. Pradeep. 2007. Screening for antimicrobial activity of weeds. Int. J. Mic. 4:1-7.

Ramirez, A., M. Moran, S. Moret, J. Gonzalez and F. Ruvalcaba. 2014. Efficiency of RAPD, ISSR, AFLP and ISTR markers for the detection of polymorphisms and genetic relationships in came de cerro (*Dioscorea* spp.). Elec. J. Bio. 17:65-71.

Seigler, D. 2003. Phytochemistry of *Acacia-sensulato*. Bio. Sys. Eco. 31:845-873.

Solano, L., M. Macia, A. Fajardo, A. Oliver and J. Martinez. 2008. Chronic *Pseudomonas aeruginosa* infection in Chronic obstructive pulmonary disease. Clin. Inf. Dis. 47:1526-1533.

Thilaga, S., R. Nair, M. Kannan and D. Ganesh. 2017. RAPD markers for screening shoot gall maker (*Betousa stylophora* Swinhoe) tolerant genotypes of amla (*Phyllanthus emblica* L.). J. Gen Eng. Bio. 15:323-330.

Vanijajiva, O. 2012. The application of ISSR markers in genetic variance detection among Durian (*Durio zibethinus* Murr.) cultivars in the Nonthaburi province, Thailand. Pro. Eng. 32:155-159.

Wickens, G. 1995. Role of *Acacia* species in rural economy of dry Africa and the Near East. FAO Conservation Guide. 27:1-176.

Wisplinghoff, H., J. Ebbers, L. Geurtz, D. Stefanik, Y. Major, M. Edmond, R. Wenzel and H. Seifert. 2013. Nosocomial bloodstream infections due to *Candida* spp. in the USA: species distribution, clinical features and antifungal susceptibilities. Int. J. Antimicrob. Agents. 43:78-81.