



## Phytochemistry, Antimicrobial, Antigiardial and Antiamoebic Activities of Selected Plants from Albaha Area, Saudi Arabia

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### Authors' contributions

This work was carried out in collaboration between all authors. Author AAA suggested the problem, proposed the outlines of the study and wrote the first draft of the manuscript. Author SMH did the phytochemical tests and performed the analysis on all samples. Author HAAGM identified and authenticated the plant species and helped in sample preparation. Author SAAR assisted in collection and identification of plant species and participated in technical editing of the manuscript. All authors reviewed the study proposal, managed the literature searches, helped in data interpretation and approved the final manuscript.

### Article Information

DOI: 10.9734/BJMMR/2016/29803

#### Editor(s):

(1) Chan-Min Liu, School of Life Science, Xuzhou Normal University, Xuzhou City, China.

#### Reviewers:

(1) Vishal W. Banewar, Government Vidarbha Institute of Science & Humanities, India.

(2) Opinde Hibert Rachuonyo, Kenyatta University, Kenya.

Complete Peer review History: <http://www.sciencedomain.org/review-history/16999>

Original Research Article

Received 29<sup>th</sup> September 2016  
Accepted 16<sup>th</sup> November 2016  
Published 23<sup>rd</sup> November 2016

### ABSTRACT

The study aimed at evaluating phytochemical components, antibacterial, antifungal, anti-giardial and anti-amoebic activities of methanol extract from *Bidens biternata*, *Silybum marianum*, *Osteospermum vaillantii*, *Achyranthes aspera* and *Asphodelus tenuifolius* plants. Phytochemical analysis revealed that *B. biternata* was rich in total phytochemical (24.3 ± 1.2%), polyphenol (16.3 ± 1.6 mg g<sup>-1</sup>) and flavonoid (9.6 ± 0.66 mg g<sup>-1</sup>) contents. The highest level of alkaloid content was detected in *A. aspera* (9.2 ± 0.08 mg/g DW) and *S. marianum* (7.6 ± 0.24 mg/g DW) extracts. *B. biternata* extract strongly inhibited the growth of *Staphylococcus aureus* with an inhibition zone equal to 29 ± 1.3 mm and showed moderate antifungal activity towards *Aspergillus niger* (16 ± 0.3 mm) and *Candida albicans* (15 ± 0.2 mm). Extract from *A. aspera* had potent anti-giardial activity against *Giardia lamblia in vitro* with an IC<sub>50</sub> = 52.91 µg/ml while the treatment of *Entamoeba histolytica* with *S. marianum* extract resulted in a marked decrease of the parasite viability with an

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IC<sub>50</sub> = 80.37 µg/ml. The antimicrobial and antiprotozoal activities of the extracted components give support and can strengthen the traditional uses of these plants.

**Keywords:** *Phytochemistry; antimicrobial activity; anti-giardial activity; anti-amoebic activity; medicinal plants.*

## 1. INTRODUCTION

For centuries, people have long been living in close association with the environment and using its flora and fauna as a source of food and medicine and many societies have their own rich plant pharmacopeias. Plants are good source of therapeutic compounds [1] and play a vital role in health care system [2]. Biologically active compounds such as polyphenols, flavonoids, tannins and alkaloids were detected in some medicinal plants [3]. The study of the bioactive compounds has become a major area of research in biological science and chemistry has led to the isolation and identification of thousands of different structures, mostly extracted from plants and more recently from microorganisms, with the animal kingdom contributing rather sparsely to the total [4-6].

*Bidens* has been used in folk medicine as anti-inflammatory, anti-malarial, anti-allergic, anti-ulcer, anti-diabetic, anti-cancer and antibacterial agent [7-10]. *Silybum marianum* is currently one of the most popular medicinal plants, and silymarin is perhaps the plant principle best researched in recent years. Extensive clinical, histological and laboratory data have confirmed the efficacy of silymarin as a hepatoprotective and antihepatotoxic agent [11,12]. There is growing interest in its anticancer and chemopreventive effects, as well as in its hypocholesterolaemic, cardioprotective, neuroactive, and neuroprotective activities [13]. *Osteospermum vaillantii* is frequently used by nomadic tribes and Arabian Bedouins in Africa and Mediterranean desert regions as a medicinal plant for treatment of fever, stomach ailments and liver disorders [14]. *O. vaillantii* have been found to contain triterpene glycosides [15] and saponins [14]. Ecdysterone, achyranthine, betaine, pentatriacontane, 6-pentatriacontanone, hexatriacontane and tritriacontane are medicinally important chemicals detected in *Achyranthes aspera*. The plant has anti-allergic, cardiovascular, nephroprotective, antiparasitic, hypoglycaemic, analgesic and antipyretic properties [16]. Crude extracts from *A. tenuifolius* had good antibacterial activity against *Proteus mirabilis* and a very good susceptibility to *Klebsiella pneumonia* and *Pseudomonas aeruginosa* [17]. In previous phytochemical

studies, Asphorodin 1, a triterpenoidal diglycoside was isolated from *A. tenuifolius*. This isolate shows a potent inhibitory activity against lipoxygenase enzyme [18].

The present work was conducted to determine some of the major classes of the secondary metabolites in five wild medicinal plants growing around Albaha area. In addition, the study aimed at assessing the antibacterial, antifungal, anti-giardial and anti-amoebic activities of the extracted components from these plants.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials and Authentication

Five wild herbaceous plants, *Bidens biternata* (Lour.) Merr. & Sherff, *Silybum marianum* (L.) Gaertn, *Osteospermum vaillantii* (Decne.) Norl., *Achyranthes aspera* L. and *Asphodelus tenuifolius* Cav. were collected in March, 2016 from different localities around Albaha area. The whole plant [shoot, root and flowers] was dried under shade for three weeks. The collected species (50 plant samples from each species) were authenticated by Dr. Haidr A. Mohammed, Department of Biology, Faculty of Science, University of Albaha. Voucher specimens were deposited at the department of Biology for further references. The plants were selected on the basis of their used in traditional medicine.

#### 2.1.1 Preparation of extracts

Dried plant materials (250 g) were ground into powders and soaked in 80% aqueous methanol (1000 ml) for 72 hours. The extracts were filtered under vacuum through Whatman's No. 1 filter paper then concentrated under vacuum using a rotary evaporator at 35°C and completely dried under a stream of air.

### 2.2 Quantitative Analysis

#### 2.2.1 Polyphenols determination

Total phenols were determined using Folin ciocalteu reagent [19]. Five ml of Folin ciocalteu reagent (1:10 diluted) and 4 ml aqueous Na<sub>2</sub>CO<sub>3</sub> (1.0 M) were mixed with 0.5 ml of each extract. After incubation for 15 min, the absorbance was read at 760 nm using a double beam (Jenway

6505) spectrophotometer (Germany). A standard curve of gallic acid (50 - 250 µg/ml) was constructed. Concentration was expressed as mg gallic acid equivalent per gram (mg GAE/g).

### **2.2.2 Flavonoids determination**

Flavonoids were determined using aluminum chloride (AlCl<sub>3</sub>) method [20]. 0.5 ml from each extract was added to mixed solution of 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1.0 M potassium acetate and 2.8 ml of distilled water. The mixture was incubated for 15 min then the absorbance was measured at 415 nm. Quercetin (10 – 100 µg/ml) was used as standard.

### **2.2.3 Alkaloids determination**

The method described by Shamsa [21] was used for determining the total alkaloids. One mg from each extract was dissolved in 2 N HCl (1 ml). After filtration, the solution was transferred to a separating funnel, 5 ml of bromocresol green and 5 ml of phosphate buffer solutions were added. The mixture was shaken with chloroform (1, 2, 3 and 4 ml) and collected in a 10 ml volumetric flask then diluted to the volume with chloroform. Atropine (20 -100 µg/ml) was used as standard. The absorbance for each sample and standard solutions was measured at 470 nm. Alkaloid content was expressed as mg atropine equivalent /g of extract.

## **2.3 Bioassay**

### **2.3.1 Determination of antimicrobial activity**

#### *2.3.1.1 Bacterial and fungal strains*

The extracts were tested for their antimicrobial activities against five bacterial and two fungal strains. The bacterial strains included, *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 35657), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 43071), *Salmonella typhi* (ATCC 19430). Two fungal strains were *Candida albicans* (ATCC 7596) and *Aspergillus niger* (ATCC 9763).

#### *2.3.1.2 Antimicrobial assay*

Antimicrobial activity was determined by disc diffusion method [22]. Briefly, the test bacteria/fungi (100 µl) were grown in fresh media (10 ml) until they reached a count of approximately 10<sup>8</sup> cells ml<sup>-1</sup> for bacteria, or 10<sup>5</sup>

cells ml<sup>-1</sup> for fungi. Bacteria were assayed on nutrient agar and Nutrient broth, while fungi on Sabouraud Dextrose Agar (Merck, USA). Microbial suspension (100 µl) was spread onto agar plates corresponding to the broth in which they were maintained. Sterilized filter paper discs (6 mm) were impregnated with 10 µl of the test sample, allowed to dry and placed onto inoculated plates. After incubation at 37 °C for 24 hours, the inhibition zones were measured in millimeters (mm). Dimethyl sulfoxide (10% DMSO) was used as negative control for bacteria and normal saline as negative control for fungi.

### **2.3.2 Antigiardial and antiamoebic assay**

#### *2.3.2.1 Parasite isolate*

*Giardia lamblia* and *Entamoeba histolytica* trophozoites were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium containing 5% bovine serum. For the assay, trophozoites were tested in their log phase of growth [23].

#### *2.3.2.2 Growth inhibition assay*

The assay was performed following the procedure described by Cedillo-Rivera and Munoz [24] using sterile 96-well white clear-bottom plates. Stock solutions were prepared with DMSO (5 mg/ml), following serial two-fold dilutions in 1.5 ml volumes of culture medium in Eppendorf tubes to afford different concentrations. The tubes were inoculated with the parasites to achieve an inoculum of 5 × 10<sup>4</sup> trophozoites/ml. As positive control, tubes with metronidazole were similarly inoculated. Tubes of culture medium with DMSO and the same inoculum were used as the negative control. After incubation for 48 h at 37°C, trophozoites were detached by chilling and 50 µl of each culture tube was subcultured into 1.5 ml of fresh culture medium and incubated for 48 h at 37°C. The final number of parasites was determined by counting in a haemocytometer, and the percentage of trophozoites growth inhibition was calculated by comparison with controls. The 50% inhibitory concentration (IC<sub>50</sub>) was defined as the concentration of the extract that inhibited growth by 50%.

## **3. RESULTS AND DISCUSSION**

### **3.1 Phytochemical Components**

Table 1 shows the percentage yields and the major secondary metabolites; polyphenol,

flavonoid and alkaloid in the studied plant species. The richest plant species of photochemical content was *B. biternata* ( $24.3 \pm 1.2\%$ ) followed by *S. marianum* ( $20.1 \pm 1.3\%$ ), *A. aspera* ( $13.3 \pm 0.26\%$ ), *A. tenuifolius* ( $8.5 \pm 0.94\%$ ) and *O. vaillantii* ( $4.2 \pm 0.64\%$ ). The amount of polyphenol, flavonoid and alkaloid was clearly varied among the plant species. The highest level of total polyphenol ( $16.3 \pm 1.6 \text{ mg g}^{-1}$ ) and flavonoid ( $9.6 \pm 0.66 \text{ mg g}^{-1}$ ) was found in extract of *B. biternata* while the amount of polyphenol in the other species was in the range of  $3.5 \pm 0.16 - 13.4 \pm 1.1 \text{ mg g}^{-1}$ . *O. vaillantii* extract had very low amount ( $1.1 \pm 0.04 \text{ mg g}^{-1}$ ) of flavonoid. Sukumaran et al. [25] have reported that total phenol content in the stem ( $0.16 \text{ mg g}^{-1}$ ) of *B. biternata* is higher than in root ( $0.15 \text{ mg g}^{-1}$ ), mature leaf ( $0.042 \text{ mg g}^{-1}$ ) and young leaf ( $0.04 \text{ mg g}^{-1}$ ). Previous phytochemical studies on *Bidens* showed that it is a rich source of bioactive constituents such as polyacetylenic glycosides, aurones, auron glycosides, *p*-coumaric acid derivatives, flavonoids and flavonoid glycosides, sesquiterpenes, phenylpropanoid glycosides, diterpenes [26–28].

*S. marianum* ( $13.4 \pm 1.1 \text{ mg g}^{-1}$ ) and *A. aspera* ( $13.2 \pm 0.92 \text{ mg g}^{-1}$ ) showed nearly equal amount of polyphenol contents. Vaknin et al. [29] proved that *S. marianum* sprouts are rich in polyphenols and demonstrate antioxidative capacity. Phytochemical studies on *A. aspera* revealed the presence of glycosides, oleanolic acid, alkaloids, saponins, indole acetic acid, polyphenol, ecdysone, and flavanoids in various parts of the plant [30]. *A. tenuifolius* contained moderate amount of polyphenol ( $11.4 \pm 0.82 \text{ mg g}^{-1}$ ) and flavonoid ( $6.3 \pm 0.05 \text{ mg g}^{-1}$ ) as compared with the other species. Faidi et al. [31] have isolated five phenolic derivatives for the first time from antimicrobial bioactive butanol extract of the narrow-leaved asphodel (*A. tenuifolius* Cav., Asphodelaceae). However, scientific interest in polyphenols isolated from plants has been aroused due to evidence indicating antioxidant,

antiinflammatory, antimutagenic, and anticarcinogenic properties [32,33].

Alkaloid content was relatively high in methanol extract of both *A. aspera* ( $9.2 \pm 0.08 \text{ mg g}^{-1}$ ) and *S. marianum* species ( $7.6 \pm 0.24$ ) as compared with the other three species ( $0.6 \pm 0.03 - 4.6 \pm 0.07 \text{ mg g}^{-1}$ ). Extracts of *O. vaillantii* ( $0.6 \pm 0.03 \text{ mg g}^{-1}$ ) and *A. tenuifolius* ( $1.6 \pm 0.05 \text{ mg g}^{-1}$ ) showed low amount of alkaloid contents. Earlier phytochemical study on *A. aspera* revealed the presence of flavonoids, saponins, steroid and terpenoids in the root and inflorescence [34,35]. Betaine and achyranthine are the principal alkaloids identified from *A. aspera* [36].

### 3.2 Antimicrobial Activity

*In vitro* antimicrobial activity of methanol extracts from five plants was investigated. The test was done on five bacterial strains, *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumonia* and *S. typhi* and two fungal strains, *C. albicans* and *A. niger* (Table 2). Methanol extracts of *B. biternata* and *A. tenuifolius* exhibited good antibacterial activity against *S. aureus* and *E. coli*, respectively, and moderate activity towards the other tested strains. In contrast, methanol extracts of *S. marianum* and *O. vaillantii* showed either no or very poor activity towards all tested pathogens. Extract from *A. aspera* displayed high activity ( $24 \pm 0.9 \text{ mm}$ ) against *C. albicans*, moderate activity towards *K. pneumonia* ( $17 \pm 0.5 \text{ mm}$ ) and *P. aeruginosa* ( $18 \pm 0.2 \text{ mm}$ ) and weak activity against *S. aureus* ( $10 \pm 0.5 \text{ mm}$ ), *E. coli* ( $12 \pm 0.6 \text{ mm}$ ) and *A. niger* ( $11 \pm 1.1 \text{ mm}$ ).

The study carried out by Reddy et al. [37] revealed that the ethanolic extract of leaves of *B. biternata* showed effective zone of inhibition against gram negative (*Escherichia coli*) and gram positive (*Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Bacillus subtilis*) strains. Faid et al. [31] who used butanol as extractive solvent demonstrated that

**Table 1. Yield percentage, total polyphenol, flavonoid and alkaloid contents in selected medicinal wild plants**

Plant extract	Yields (%)	Total polyphenols (mg /g DW)	Flavonoids (mg/g DW)	Alkaloids (mg/g DW)
<i>B. biternata</i>	$24.3 \pm 1.2$	$16.3 \pm 1.6$	$9.6 \pm 0.66$	$4.6 \pm 0.07$
<i>S. marianum</i>	$20.1 \pm 1.3$	$13.4 \pm 1.1$	$6.3 \pm 0.05$	$7.6 \pm 0.24$
<i>O. vaillantii</i>	$4.2 \pm 0.26$	$3.5 \pm 0.16$	$1.1 \pm 0.04$	$0.6 \pm 0.03$
<i>A. aspera</i>	$13.3 \pm 0.94$	$13.2 \pm 0.92$	$3.3 \pm 0.07$	$9.2 \pm 0.08$
<i>A. tenuifolius</i>	$8.5 \pm 0.64$	$11.4 \pm 0.82$	$3.2 \pm 0.08$	$1.6 \pm 0.05$

**Table 2. Anti-microbial activity of methanol extracts from selected medicinal wild plants against five bacterial and two fungal strains**

	Inhibition zone (mm)						
	Bacterial strains					Fungal strains	
	S	E	K	SA	P	C	A.
<i>B. Biternata</i>	29 ± 1.3	17 ± 0.4	18 ± 0.7	16 ± 0.2	17 ± 0.8	15 ± 0.2	16 ± 0.3
<i>S. Marianum</i>	5 ± 0.4	9 ± 0.2	0	6 ± 0.3	8 ± 0.2	0	0
<i>O. Vaillantii</i>	7 ± 0.3	0	6 ± 0.2	0	0	0	0
<i>A. Aspera</i>	10 ± 0.5	12 ± 0.6	17 ± 0.5	12 ± 0.4	18 ± 0.2	24 ± 0.9	11 ± 1.1
<i>A.tenuifolius</i>	15 ± 1.2	28 ± 1.5	17 ± 1.2	19 ± 0.9	17 ± 0.8	16 ± 0.6	17 ± 0.4
DMSO	0	0	0	0	0		
Normal saline						0	0

Key: S = *Staphylococcus aureus*, E = *Escherichia coli*, P = *Pseudomonas aeruginosa*, K = *Klebsiella pneumonia*, SA = *Salmonella typhi*, C = *Candida albicans*, A = *Aspergillus niger*. No activity = 0, low activity = < 15 mm, moderate activity = 15 – 20 mm, high activity = 21 – 25 mm and very high activity = > 25 mm

extracts of *A. tenuifolius* exhibited significant antifungal activity against *Candida albicans*, *Candida parapsilosis* and *Candida krusei* and a considerable level of antibacterial activity towards *Escherichia coli* (minimum inhibitory concentration (MIC) = 729 µg/ml) and *Pseudomonas aeruginosa* (MIC = 156 µg/ml).

Menghani et al. [38] studies on petroleum ether, benzene, chloroform, ethyl acetate and methanol extracts of *A. tenuifolius* proved that the plant has potential antimicrobial activities. Several investigators have also reported that the different parts of the plant *A. aspera* exhibited equipotent activity against fungal strains *Candida albicans*, *Aspergillus flavus*, *Cryptococcus neoformans*, *fusarium* sp. and *Sclerouum* sp. [39-41].

### 3.3 Antigiardial Activity

Table 3 displays the results of *in vitro* anti-giardial activity of methanol extracts against *G. lamblia* trophozoites as compared to the positive control. The tested plant extracts at all levels of concentrations inhibited growth of *Giardia* trophozoites with different rates. Extracts of *A. aspera* had high activity while the other extracts showed moderate to weak activity against the parasite. At concentrations 500, 250 and 125 µg/ml, extract of *A. aspera* exhibited 89.7, 85.4 and 74.5% cell death, respectively. The calculated IC<sub>50</sub> value (52.91 µg/ml) of this extract was much lower than that of the other extracts.

According to the criteria of Amaral et al. [42], who established extracts with IC<sub>50</sub> ≤ 100 µg/ml as highly actives; extract of *A. aspera* was very active towards the protozoan, being as an outstanding when compared with the other

extracts. The results obtained by Zhang et al. [43] demonstrated that an aqueous ethanol extract of *Artemisia argyi* inhibited the aminoacylation activity of LeuRS from *G. lamblia* (GILeuRS). Johns et al. [44] who studied 36 plant species representing different families have revealed that 21 species extracts were lethal or inhibited growth of *Giardia* trophozoites at 1000 ppm; 7 species were lethal at 500 ppm concentration.

**Table 3. Anti-giardial activity of methanol extract from selected medicinal wild plants**

	Concentration (µg/ml)			
	Mortality (%)			
	500	250	125	IC <sub>50</sub>
<i>B. biternata</i>	50.3	44.1	28.6	224.64
<i>S. marianum</i>	66.5	56.7	44.8	161.79
<i>O. vaillantii</i>	64.7	55.9	40.1	172.74
<i>A. aspera</i>	89.7	85.4	74.5	52.91
<i>A. tenuifolius</i>	51.0	42.5	30.3	219.82
Positive control		95.2		

### 3.4 Antiamoebic Activity

Methanol extracts of the studied plants were screened for *in vitro* activity against *E. histolytica*. Crude extracts showed different degrees of antiamoebic activity. At high concentration level (500 µg/ml) the mortality percentage was in the range of 59.1 – 70.0% while at low level (100 µg/ml) it was ranging from 30.1 – 58.2%. The calculated IC<sub>50</sub> values ranging from 80.37 to 344.62 µg/ml. The strongest level of inhibition was recorded by extract from *S. marianum* with an IC<sub>50</sub> = 80.37 µg/ml. Referring to Amaral et al. [42] criteria, this finding suggested that

*S. marianum* methanol extract could have ability for killing *E. histolytica*.

However, forty-five crude extracts from selected medicinal plants were screened by Tona et al. [45] against *E. histolytica*. The results indicated that 35 species demonstrated an antiamebic activity and 10 were inactive. In another study, benzene and ethyl acetate extracts from the root bark of *Adina cordifolia* strongly inhibited the growth of *E. histolytica* trophozoites with IC<sub>50</sub> values of 2.92 and 2.50 mg/ml, respectively [46].

**Table 4. Anti-amoebic activity of methanol extracts from selected medicinal wild plants**

	Concentration (µg/ml)			
	Mortality (%)			
	500	250	125	IC <sub>50</sub>
<i>B. biternata</i>	60.2	51.6	42.2	237.62
<i>S. marianum</i>	73.0	61.2	58.2	80.37
<i>O. vaillantii</i>	60.1	40.0	30.1	329.62
<i>A. aspera</i>	69.4	55.3	40.5	196.37
<i>A. tenuifolius</i>	59.1	49.2	37.2	344.62
Positive control		95.2		

#### 4. CONCLUSION

Phytochemical analysis revealed variation among the studied species in their chemical components. *B. biternata* contained high amount of total phytochemicals, polyphenols and flavonoids. Methanol extracts from *B. biternata* have been identified to possess antibacterial and antifungal activities. *A. aspera* and *S. marianum* extracts showed remarkable anti-giardial and anti-amoebic activities against *G. lamblia* and *E. histolytica* trophozoites, respectively. The obtained results may offer a scientific basis for traditional uses of these plants for treatment of many diseases. In addition, these findings demonstrated that the wild medicinal plants of Albaha area have potentials as source of antimicrobial anti-giardial and anti-amoebic agents. However, the obtained results may strengthen the traditional uses of these plants in folk medicine.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### ACKNOWLEDGEMENT

The present research is a part of a project funded by the Deanship of Scientific Research, Albaha University, KSA (Grant No. 70 - 1436). Financial support of the Deanship is gratefully acknowledged.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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