Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(4): 1317-1321

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Total phenolic and total flavonoid contents of different solvent extracts of *Bassia muricata*(L.) Asch. and evaluation of antibacterial and antioxidant activities

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ABSTRATCT

This study aimed to examine the polyphenol and flavonoid contents of different solvent extracts of Bassia muricata with antioxidant activity by measuring the radical 2,2-diphenyl-1-picylhydrazyl scavenging activity and antibacterial activity by using disc diffusion method. The total phenol content amongst the extracts were significantly higher in butanol extract (199.80 µg GAE mg^{-1}) and decreased in the following order: butanol extract > ethanol extract > ethyl acetate extract. Antioxidant activity was also highest for butanol extract ($p<0.05$). Antioxidant activity was generally found to increase with total phenol content (TFC) and total flavonoid content (TFC). Bassia muricata extracts have shown significantly antibacterial activity against various gram-positive and gram-negative bacteria. The highest antibacterial activity has shown with butanol extract.

Keywords: *Bassia muricata*, Antibacterial, Antioxidant, Total Phenolic Content, Radical scavenging potential.

INTRODUCTION

The plant *Bassia muricata* species belongs to the *Chenopodiaceae* family. It is an erect or decumbent villous woody annual, branched from the base, up to 80 cm in height; Leaves lanceolate, linear, 10-15 mm long, 1.5-2 mm wide. Flowers are variable in number 1-3, perianth sparsely villous; fruiting perianth with needle-like spines, spines are glabrous, straight, as long as or over twice the diameter of the fruit. It is flowering in spring (March-April) [1-4]. It is used in folk medicine to treat renal and rheumatic diseases and possess different degrees of anti-inflammatory, analgesic and antipyretic effects as well as antispasmodic property [5]. The species is a common herb in sandy soils and at the margins of desert roads. It is recorded in Iran, Palestine, Arabia and North Africa.

In previous phytochemical studies, three metabolites, 3,4-dimethoxytoluene, 3′-methylquercetin and a new flavonoid glycoside, 3-*O*-[α-L-arabinopyranosyl-(1→2)-L-α-arabinopyranosyl)]-3′-methylquercetin were isolated from *Bassia muricata*. The last two compounds have been shown high DPPH radical scavenging [5]. In other study, two acylated flavonoid glycosides quercetin-3-*O*-(600 caffeoyl)-sophoroside and quercetin-3-*O*-(600-feruloyl)-sophoroside have been isolated together with two known flavonoid glycosides quercetin-3-*O*-sophoroside and quercetin-3,7-*O*-b-diglucopyranoside, as well as four known triterpenoidal saponins, oleanolic acid 3-*O*-b-glucopyranoside, chikusetsusaponin IVa, chikusetsusaponin IVa methyl ester and oleanolic acid-3,28-b diglucopyranoside [6].

Therefore, the objective of this study was to compare the TPC, TFC, antiradical scavenging and antibacterial activities of the extracts of different solvent extractions from aerial parts of *B. muricata*.

EXPERIMENTAL SECTION

2.1. Plant material

The aerial parts of *B. muricata* were collected in Algerian Sahara during the flowering period in April 2015 near Trifaoui, El-Oued, Algeria (33°25'0"N, 6°55'0"E) at 77 m altitude and taxonomic identification of the plant was confirmed by Dr. Atef CHOUIKH. The collected plant material was air-dried in darkness at room temperature for three weeks. A voucher specimen was deposited in the herbarium of the Faculty of Life and Natural Sciences of El Oued University (*B. muricata* voucher number CAK 16).

2.2. Preparation and extracts

Total ethanol extract of *B. muricata* was prepared by maceration technique, the dried and powdered aerial parts of the plant (14.6 g) were macerated with 200 mL of ethanol at room temperature (25°C) 3 times (24 hours × 3). After filtration, the extract was concentrated using a rotary evaporator at a maximum temperature of 45°C, the residuals obtained were divided, a half part was stored in a freezer at -4°C until further study. The second half (2.20 g) of the ethanol dry extract was dissolved in 200 mL hot water. The water solution was filtered and partitioned with ethyl acetate and n-butanol. Organic layers of each of the two solvents were filtered and evaporated under vacuum to dryness to give 85mg ethyl acetate fraction, 86 mg butanol fraction. The insoluble white part of the aqueous ethanolic extract after partition between the above two solvents was not a part of our study. carefully collected, filtered, air-dried, weighed (residue 2: 0.53 g) and kept in the fridge (0°C) together with the other extracts, under nitrogen atmosphere, until use. The dried samples were kept in the freezer at -4°C. until use.

2.3. Determination of total phenolic content (TPC)

The concentration of phenolic content in Methanol extract was expressed as micrograms of Gallic acid equivalents, determined with Folin–Ciocalteu reagent (FCR), according to the method of Slinkard and Singleton [7]. 1 mL of the solution containing 1 mg of the tested extract in methanol was added to 46 mL of distilled water and 1 mL of FCR, and mixed thoroughly. After 3 minutes, 3 mL of sodium carbonate solution (2%) were added to the mixture and shaken intermittently for 2 hours at room temperature. The absorbance was read at 760 nm. The concentration of phenolic compounds was calculated according to the following equation that was obtained from the standard pyrocatechol graph:

$$Absorbance = 0.001\ \mu g\ Gallic\ acid + 0.0095 \quad (r^2 = 0.988)$$

2.4. Determination of total flavonoid content (TFC)

Measurement of flavonoid concentration of the extract was based on the method described by Park et al. in 1997 with a slight modification [8], and result was expressed as quercetin equivalents. An aliquot of 1 mL of the solution (contains 1 mg of extract in methanol) was added to test tubes containing 0.1 mL of 10% aluminium nitrate, 0.1 mL of 1 M potassium acetate and 3.8 mL of ethanol. After 40 minutes at room temperature, the absorbance was determined at 415 nm. Quercetin was used as a standard. The concentration of flavonoid compounds was calculated according to following equation that was obtained from the standard quercetin graph.

$$Absorbance = 0.0046\ \mu g\ Quercetin + 0.0149 \quad (r^2 = 0.996)$$

2.5. Determination of DPPH radical scavenging activity

The free radical scavenging activity of the essential oil and methanol extract were determined by the DPPH assay described by Blois with slight modification [9, 10]. In its radical form DPPH absorbs at 517 nm, but on reduction by an antioxidant or a radical species its absorption decreases. Briefly, a 0.1 mmol L^{-1} solution of DPPH in methanol was prepared and 4 mL of this solution was added to 1 mL of samples solution in methanol at different concentrations. Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

The capability to scavenge the DPPH radical of an antioxidant was calculated using the following equation:

$$DPPH\ scavenging\ effect\ (\%) = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

The sample concentration providing 50% free radical scavenging activity (IC_{50}) was calculated from the graph of DPPH Scavenging effect percentage against sample concentration. Vitamin C was used as antioxidant standard for comparison of the activity.

2.6. Evaluation of antibacterial activity

Five strains Gram negative bacteria *Pseudomonas aeruginosa, Vibrio cholerae, Salmonella enterica, Serratia marcescen* and *Escherichia coli* and four strains Gram positive bacteria *Micrococcus luteus, Staphylococcus epidermidis, Enterococcus faecalis* and *Bacillus anthracis* were used to assess their susceptibility to extracts of *B. muricata*. The antibacterial activity was determined by the disc diffusion method using Mueller Hinton agar (MHA) [11]. Inoculated each bacterial strain transferred to a small bottle of tryptic soy broth (TSB) and incubated at 37^{o}C for 24 hours. The bacterial strain suspension was adjusted to 0.5 McFarland turbidity standard (1 x 10^8 CFU/mL). Then, inoculated each strain was spread on MHA plates. The dried sample of the extract was dissolved in dimethyl sulfoxide (DMSO) to a concentration of 2 mg/mL. Sterilized filter paper discs were impregnated with 20 µL of extract to give a final of 40 µg/disc and the discs were placed on the agar surface of MHA. For negative controls, 20 µL of DMSO was added to a sterile paper disc and antibiotic disc of Amoxicillin (25 µg/disc) was used as positive control. The inoculated plates were incubated at 37^{o}C for 24 hours. Antibacterial activities were determined by measurement of the inhibition zone diameter (mm) around each paper disc.

2.7. Statistical analysis

All analyses were carried out in triplicate. Data are expressed as mean ± standard deviation (SD). Differences were estimated by one-way analysis of variance (ANOVA) followed by least significance difference (LSD) test. Probability values of less than 0.05 were considered statistically significant. Pearson correlation coefficient (R^2) and p-value were used to show correlations and their significance.

RESULTS AND DISCUSSION

3.1. Total phenolic content (TPC)

Lipid oxidation is stabilized with polyphenolic compounds having antioxidant activity [12]. Polyphenolic compounds are also indispensable due to having inhibitory effects on carcinogenesis and mutagenesis in humans. According to Tanaka et al. 1.0 g daily is suggested to ingest from foods [13]. Therefore, the amount of phenolics in the studied extracts was measured by Folin–Ciocalteu method. The concentration of phenolics in the extract was expressed as micrograms of gallic acid equivalents per milligrams of the extract. Table 1 shows Gallic acid equivalents of total phenolics of *B. muricata* extracts. Content of phenolic compounds in the extracts varied from 18.22 ± 4.44 µg GAE mg^{-1} in ethyl acetate extract 199.80 ± 0.55 µg GAE mg^{-1} in butanol extract. As displayed in Table 1, the total phenolic content (TPC) of the extracts can be ranked as: Butanol extract > Ethanol extract > Ethyl acetate extract. A similar level of diversity in phenolic contents was seen by Bouaziz et all [14] who examined hexane, ethyl acetate, methanol and water extracts of *B. muricata* collected from Douz region of Tunisia.

3.2. Total flavonoid content (TFC)

Flavonoids are the major components of the phenolic compounds. The total flavonoid content (TFC) extracts were estimated by using aluminum nitrate colorimetric assay. The concentration of flavonoids in the extract was expressed as micrograms of quercetin equivalents per milligrams of the extract (Table 1).The total flavonoids ranged from13.62 ± 2.94to 39.21 ± 5.19µg QE mg^{-1}. Ethanol extract had the highest TFC value. Whereas, ethyl acetate extract had lowest value.

Table1. Extraction yield, total phenolic and flavonoid of *B. muricata* extracts

Samples	Yield (%)	Total phenolic content (µg GAE mg^{-1})	Total flavonoid content (µg QE mg^{-1})
Ethanol extract	15.01	46.55 ± 1.66^{b}	31.76 ± 0.49^{b}
Butanol extract	1.18	199.80 ± 0.55^{a}	39.21 ± 5.19^{a}
Ethylacetate extract	0.58	18.22 ± 4.44^{b}	13.62 ± 2.94^{c}

value are means± standard deviation, n = 3. Means followed by the same letter are not significantly different at α = 0.05 level by LSD (least significant difference) test.

3.3. DPPH radical scavenging activity

Lipid oxidation is stabilized with polyphenolic compounds having antioxidant activity [12]. Polyphenolic compounds are also indispensable due to having inhibitory effects on carcinogenesis and mutagenesis in humans. According to Tanaka et al. 1.0 g daily is suggested to ingest from foods [13]. Therefore, the amount of phenolics in the studied extracts was measured by Folin–Ciocalteu method. The concentration of phenolics in the extract was expressed as micrograms of gallic acid equivalents per milligrams of the extract. Table 1 shows Gallic acid equivalents of total phenolics of *B. muricata* extracts. Content of phenolic compounds in the extracts varied from

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Table2. DPPH scavenging activity of *B. muricata* extracts

Samples	DPPH activityIC50 (µg/mL)
Ethanol extract	181.79 ± 3.80[b]
Butanol extract	50.57 ± 3.56[a]
Ethylacetate extract	975.14 ± 61.53[c]
Vitamin C	62.23 ± 0.94[a]

value are means± standard deviation, n = 3. Means followed by the same letter are not significantly different at α = 0.05 level by LSD (least significant difference) test.

Among the three extracts, the butanolic extract showed a significant phenolic content and radical scavenging activity. In general, extracts with a high radical scavenging activity showed a high phenolic content as well, also good correlations found among them (Figure 1). A direct correlation between radical scavenging activity and phenolic content of the extracts was demonstrated by linear regression analysis. The correlation coefficient between total phenolics and DPPH found to be 0.99. In general, the ethyl acetate fraction showed the highest TPC and the highest radical scavenging activity.

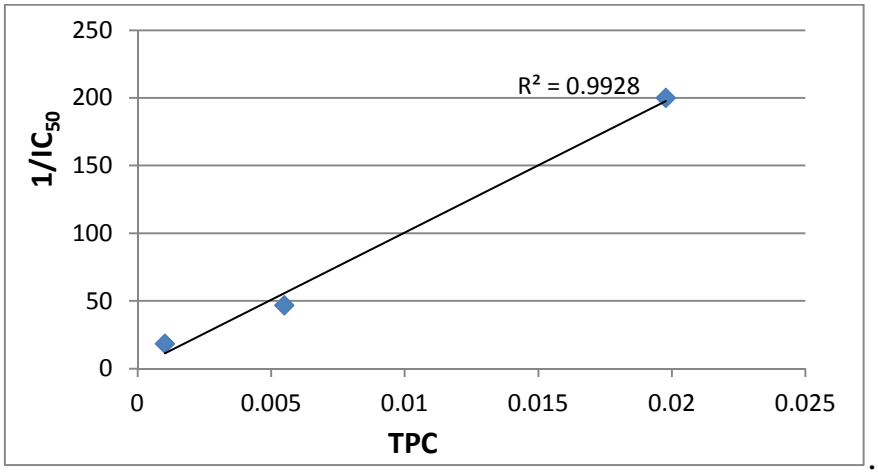

Figure 1. Correlation between total phenolics and DPPH scavenging activity

3.4. Antibacterial activity

The antibacterial activities of the aerial parts extract from *B. muricata* using different solvent extractions were. Butanol extract showed significantly higher antibacterial activity against various gram positive and negative bacteria (P<0.05). The Butanol extract had the best antibacterial activity than other solvent extracts, the diameter of inhibition zone ranged from 7 to 9 mm were presented in compared to the positive control (Amoxicillin 25 µg/disc) the results showed in Table 3.Inaddition, all of the negative control (DMSO) had no inhibitory effect on the bacteria growth. All extracts inhibited growth of five bacterial strains. The sensitive bacteria to the extract were *Escherichia coli*, *Micrococcus luteus*, *Salmonella enterica*, *Vibrio cholera* and *Pseudomonas aeruginosa*. The strains of *Serratia marcescen*, *Staphylococcus epidermidis* and *Bacillus anthracis*. Whereas, *Enterococcus faecalis* inhibited only with butanol extract. However, to the best of our knowledge, there has been no scientific report on antibacterial activity of the extracts of *B. muricata* yet.

Table 3. Antimicrobial activity of *B. muricata*extracts

Microorganisms	Ethanol extract	Butanol extract	Ethylacetate extract	Amoxicillin
Pseudomonas aeruginosa	8	7	7.5	7
Vibriocholerae	7	9	7	12
Salmonella enterica	7	7	8.25	15.5
Serratiamarcescen	0	0	0	0
Escherichia coli	9.5	7	7.5	10
Micrococcusluteus	8	8	9.5	0
Staphylococcus epidermidis	0	0	0	22
Enterococcusfaecalis	0	8	0	0
Bacillus anthracis	0	0	0	0

CONCLUSION

A marked antimicrobial and radical scavenging effect of different solvent extracts of *B. muricata* was demonstrated in the present study. It is observed the bioactivities bioactivities of extracts could be ascribed to the presence of secondary metabolites present in the extracts in particular phenolic compounds. A direct correlation was observed between the content of total phenolics and radical scavenging activity of extracts. The plant appears promising resources of the bioactive agents which can be suggested as a natural additive in food and pharmaceutical industries. Further studies are necessary to evaluate the origin of the activity.

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