



RESEARCH PAPER

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Phytochemical screening, antibacterial and antioxidant activity of *Monoteheca Buxifolia* stem

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Abstract

The aim of the current study was to screen the phytochemicals present in the various fractions of the plant using the biochemical tests and to evaluate the antibacterial and antioxidant potential of the different fractions of *Monoteheca buxifolia* stem using Well method and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. The antibacterial activities of these fractions were checked against *Escherichia coli*, *Pseudomonas aeruginosa*, *Erwinia carotovora*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*. These results obtained revealed that this plant have good antibacterial and antioxidant potential and very important from medicinal point of view.

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Introduction

Large numbers of natural products have been isolated from the medicinal plants. These include flavonoids, tannins, sterols, alkaloids, triterpenoids, essential oils etc. These natural constituents play a key role in the field of medicines (Basu and Rastogi, 1967, Edeoga *et al.*, 2005, Cseke *et al.*, 2016, Harborne, 1998). These biologically active constituents can be derived from different parts of plants like barks, leaves, flowers, roots, fruits and seeds etc. (Oliver-Bever, 1986).

Monothea buxifolia is evergreen tree and traditional medicinal plant having broad leaves. It belongs to family *Sapotaceae* (Shah *et al.*, 2012a). This species is distributed in the mountains of Afghanistan and the main center is Northern Pakistan. In Pakistan it is commonly found in Gorakh Hills, Zhob, Loralai, Kohat, Drosh Chitral, Kala Chitta Hills, Dir, and Attock District (Nasir & Ali, 1972). The family *Sapotaceae* is widely studied for antioxidant (Jan *et al.*, 2013), CNS depressant (Nasrin *et al.*, 2011), anthelmintic (Koné *et al.*, 2012) antimicrobial (Kueté *et al.*, 2006; Marwat *et al.*, 2011), anti-inflammatory (Araujo-Neto *et al.*, 2010; Purnima *et al.*, 2010), antipyretic (Shekhawat *et al.*, 2010) and antinociceptive activities [(Shekhawat *et al.*, 2010); Karmakar *et al.*, 2011) in various in vitro and in vivo experimental models.

In traditional medicine, *M. buxifolia* fruit is used as laxative, antipyretic, purgative, vermifugal, hematinic and for the management of gastro-urinary disorders (Shah *et al.*, 2013; Ullah *et al.*, 2010; Rehman *et al.*, 2013; Murad *et al.*, 2013) The leaves of *M. buxifolia* contain flavonoids, saponins, terpenoids, anthraquinones, cardiac glycosides, tannins, reducing sugars, and poly-phenolic compounds (Rehman *et al.*, 2013) Recently, two novel compounds, buxilide as pyrone and buxifoline-A as alkaloid were isolated from the ethylacetate fraction of *M. buxifolia* fruit (Ullah *et al.*, 2016).

Flavonoids and poly-phenolic compounds are reported to possess potent analgesic and anti-inflammatory properties.

Previously, the in vitro antioxidant activity of this fruit has been evaluated and has proved to exhibit potent antioxidant properties (Jan *et al.*, 2013). Moreover, *M. buxifolia* fruit also possess inhibitory potential against urease enzyme (Ullah *et al.*, 2016). The whole plant of *M. buxifolia* is frequently used in the preparation of herbal drugs. The fruit is laxative and also helps in digestion as well as used for the treatment of urinary tract diseases (Ullah *et al.*, 2013). The fruit is a rich source of vitamin C and other important photochemical. The stem part of the plant is less investigated for photochemical and various biological activities (Anwar *et al.*, 2007). This Plant is being used for medicinal purposes by local people since ancient times. It is a well-recognized fact that this plant serves a potent medicine for curing various diseases like fever and diabetic (Jan *et al.*, 2013). The current study was conducted to investigate the phytochemicals and bioactivities of various fractions of *M. buxifolia* stem.

Material and methods

Collection and drying of plant materials

The *M. buxifolia* plant was collected in March, 2014 from Shamoza (District Buner) Khyber Pakhtunkhwa, Pakistan in blossoming season. Plant was recognized by the Department of Botany, Abdul Wali Khan university Mardan (AWKUM). The sample was washed away properly with de-ionized water for eliminating dirt, dust and other possible contamination. The plant was washed and dried at room temperature for fifteen days and then crumpled into abrasive powder using grinder and then stored in clean, dried plastic bags for further processing.

Extraction and fractionation

The shad dried plant powder was chopped and soaked in methanol for 10 days with regular shaking five times a day. The filtrate was evaporated under reduced pressure by using Vacuum Rotary Evaporator keeping the temperature at 40°C to give crude extracts. Extracts was made dried and weighed. The crude extracts of *M. buxifolia* stem was suspended in water and different fractions were separated successively with *n*-hexane, chloroform, ethyl acetate and methanol polarity gradient wise for obtaining *n*-hexane soluble, chloroform-soluble, ethyl acetate-soluble and methanol soluble fractions respectively.

From each fraction the respective solvent was evaporated by rotary evaporator keeping definite temperature for each solvent. By this method, sticky deposit of each fraction was acquired.

Phytochemical Screening

Phytochemical screening of various fractions of *M. buxifolia* stem was performed using standard procedures as described by Sofowora, (1993), Trease and Evans, (1989) and Harborne (1973).

Alkaloids

To check out alkaloids roughly 0.2g of each fraction extract was heat up with 2% Sulfuric acid for about 2 minutes. Then it is filtered and adds few drops of dragendroffs reagent. If orange red precipitation is obtained it testified positive result of alkaloids.

Tannins

Small portion of each fraction extract was mixed with water and warmed up by using water bath and carried out filtration with the addition of ferric chloride. If dark green solution is obtained it shows the presence of tannins.

Anthraquinone

0.5g of the fraction extract was dissolved with 10% HCl and boiled it for some time; the filtration is carried out and allowed to cool. Add chloroform (eq. volume) to the filtrate. Add few drops of 10% ammonia to it and heated. If rose pink color appears, it confirms the presence of anthraquinone.

Glycosides

Each fraction extract was treated with hydrochloric acid and it is neutralized by sodium hydroxide. Add few drops of ferric chloride A and B. The red precipitation show glycoside.

Reducing sugar

Each fraction extract was mixed with water (Dilute) and filtered. The filtrate was boiled with few drops of ferric chloride A and B for some time. Orange red Precipitation indicates positive result in the respective extract.

Saponine

Some portion of the respective fraction extract was dissolved in 5ml distilled water. It is heated up to boiling foaming indicate saponine in that extract.

Flavonoids

About 0.2g of the fraction extract was mixed with dilute sodium hydroxide and hydro chloric acid was added. If yellow solution appears which, turn colorless it confirms the presence of flavonoids in the respective extract.

Terpenoids

0.2g of each fraction extract was dissolved with about 2ml of chloroform. Add few drops of 3ml concentrated sulfuric acid to form a layer. Reddish brown color confirms terpenoids in the sample.

Antibacterial Activity

Test Microorganisms

The organisms selected for antimicrobial activity were *Escherichia coli*, *Pseudomonas aeruginosa*, *Erwinia arotovora*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*.

Preparation of media

Media of nutrient agar was primed in conical flask according to the instructions provided by the maker. The media along with the Petri dishes, pipette and metallic rod were pasteurized in autoclave for 15 minutes at 121°C and 15 psi pressure. The media were then poured into Petri dishes under aseptic condition (Laminar flow hood) and let them for solidification for about 25 minutes.

Well assay method

After solidification of media Wells of 6mm were dug in media by using sterilized plastic borer. Each well was given a specific number. Bacterial culture equivalent to 10⁴ to 10⁶ CFU (colony forming unit) was vaccinated/marked on the surface of the solidified media. Stock solutions of each fraction in DMSO at concentration of 5mg/mL were primed from stock solution was added into corresponding wells. Petri dishes were then kept in incubator at 37°C for incubation. The areas of reserve were measured after 24 hours of incubation. Ciprofloxacin was used as standard and as a positive control while DMSO was used as used as a negative control. The areas of inhibition of *n-hexane*, chloroform, ethyl acetate and methanol fractions were matched with areas of inhibition of standard medicines ciprofloxacin. The amount of each evolution well was measured.

DPPH radical scavenging assay

The reaction mixture containing various concentrations 10, 30, 50, 90, 150 and 200 μ M of various fractions were mixed with 85 μ M methanolic solution of DPPH radical and the reaction mixture is then incubated for 30 min at room temperature and after this measure the decrease in absorbance by the UV spectrophotometer. Ascorbic acid taken in the same concentrations as that of the fractions act as a positive control. To find out the percent inhibition of the fractions as well as the Ascorbic acid using the following formula.

$$\text{DPPH Inhibition effect(\%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where,

A_c = Absorbance reading of the control

A_s = Absorbance reading of the sample

Results and discussions*Results of Phytochemical Screenings*

The results of the phytochemical screening of the selected plant are given in Table 1.

Results of Antibacterial Activity of M. buxifolia stem

In the present study, the antibacterial activity of various fractions of *M. buxifolia* stem i.e. n-hexane, chloroform, ethyl acetate, and methanol was determined. All the fractions of *M. buxifolia* stem were found active against all bacterial strains. Ciprofloxacin was used as a standard. Among the fractions the high activity was shown by the ethyl acetate fraction, while the low activity was shown by the n-hexane fraction. The zones of inhibition formed by the fractions are given in (Table 2).

Table 1. Phytochemical screening of *M. buxifolia* stem.

Sample	Alkaloids	Tannins	Anthraquinones	Cardiac glycosides	Reducing Sugar	Saponins	Flavonoids	Terpenoids
n-hexane	-	-	-	+	+	+	-	-
Chloroform	-	+	+	+	+	+	+	+
Ethyl Acetate	-	+	-	-	+	+	+	+
Methanol	-	+	+	+	+	+	+	+
Water	-	-	+	-	+	+	+	+

Key. + Present - Absent.

Table 2. Results of Antibacterial Activity

Microorganism	Zone of inhibition (mm)				
	n-hexane Fraction	Chloroform Fraction	Ethyl acetate Fraction	Methanol Fraction	STD
<i>Escherichia coli</i>	12	13	17	17	15
<i>Pseudomonas aeruginosa</i>	15	15	20	20	35
<i>Erwinia carotovora</i>	8	7	20	21	11
<i>Salmonella typhi</i>	13	17	22	16	21
<i>Klebsiella pneumoniae</i>	7	18	24	18	30
<i>Staphylococcus aureus</i>	8	18	25	21	16
<i>Bacillus subtilis</i>	15	22	28	12	15

DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of methanolic solutions of the different fractions of *M. buxifolia* stem were used with DPPH radicals in comparison with Ascorbic acid (Liang *et al.*, 2010, Cheng *et al.*, 2010, Mishra *et al.*, 2012). Among the fractions of the plant stem the highest scavenging ability was shown by ethyl acetate fraction whose IC_{50} values are of

194.24 \pm 7.36 μ M while the lowest scavenging ability was observed by chloroform which shows the IC_{50} values of 686.61 \pm 8.75 μ M.

These activities were concentration dependent and maximum DPPH scavenging ability was observed at higher concentrations as shown in Table 3.

Table 3. % Inhibition of DPPH Radical Scavenging.

Drugs	%Inhibition Mean(n=3)					IC ₅₀ (μM) ± SEM
	12.5 (μM)	25 (μM)	50 (μM)	100 (μM)	200 (μM)	
<i>n</i> -hexane	35.08	51.23	50.00	69.43	76.04	428.50±7.34
Chloroform	8.83	24.00	22.62	43.58	58.68	686.61±8.75
Ethyl acetate	9.87	14.35	17.64	33.39	49.83	194.24±7.36
Methanol	5.52	19.30	15.15	30.37	37.62	265.99±5.65
Ascorbic acid	62.43	66.58	71.94	76.98	80.07	35.64±9.76

Conclusion

In the current study the stem part of *M. buxifolia* were investigated to explore its medicinal importance. The results obtained revealed that all the fractions of *M. buxifolia* stem were found active against all bacterial strains. While the highest DPPH scavenging activity was shown by the ethyl acetate fraction with the IC₅₀ values of 194.24±7.36μM.

The results obtained revealed that this plant is very important from biological point of view and it need further investigation to be carried out to explore its hidden medicinal potential.

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Conflicts of interest

Authors have none to declare.

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