Abstract—The current study was focused on the phytochemical investigation and pharmacological activities of the various fractions (methanolic crude, defatted methanolic, n-hexane, dichloromethane, ethylacetate and water) of *Heliotropium curassavicu*m. The various plant extracts were examined for the total phenolic contents by taking Gallic acid as a standard, antioxidant scavenging assay by using DPPH (2,2-diphenyl-1-picrylhydrazly) & ABTS [2, 2-azinobis (3-ethylbenzthiazoline-6-sulphonic acid)] free radicals, antifungal assay by using Terbinfine solution in DMSO as positive control and pure DMSO as negative control and in-vitro antibacterial activity. Methanolic crude extract showed high phenolic contents and significant antioxidant activity (both DPPH & ABTS). All other fractions were also found to have significant potential.

Keywords—*H. curassavicu*m, phytochemical investigation, pharmacological activities, DPPH, ABTS.

I. INTRODUCTION

The genus *Heliotropium* belongs to family Boraginaceae, and comprises about 250 to 300 species. The genus mainly consists of flowers bearing plants, and is local to America and stretches from Argentina to Canada. *H. curassavicu*m L. is a persistent herb and comprises of fleshy stem, with spade or oval-shaped leaves and white colored five lobed curved flowers (Gokulnath et al., 2014; Weiss, 1995). A large diversity of chemical compounds has been isolated from *H. curassavicu*m which are used effectively against infections from predators such as insects, fungi and bacterial infections and to cure various human diseases (Yogamooorthi and Sathiyapriya, 2006). *H. curassavicu*m has been traditionally used for ulcers, wounds; local inflammations cure gonorrhea, erysipelas, enema constipation, edema, bacterial infections cancer and diabetes (Satyavani et al., 2013). The plant has been used as curative for wounds and sores by Indians of south-west American (Subramaniam et al., 1982; Krochmal et al., 1954). In Argentina it is used for the treatment of arteriosclerosis, rheumatism and to progress blood circulation to usual (Davicino et al., 1988). The presence of tannins, protein, phenol, carbohydrates, saponins, terpenoids and alkaloids have been reported from *H. curassavicu*m (Karthishwaran et al., 2010; Choudhary et al., 2013; Guntern et al., 2001; Catalfamo et al., 1982, Singh et al., 2002). Most of the constituents of these plants were found to have various biological activities, including anti-tumoural, anti-microbial, and anti-viral effects (Goyal and Sharma, 2014).

A large number of human, animal and plant diseases are caused by pathogenic microbes (fungi and bacteria). The deaths of higher organisms have been caused mainly by Bacteria and fungus infections (Sardi et al., 2013). Determination of antibacterial, antifungal and antioxidant activities gives an insight about the pharmacological significance of biological compounds and plant extracts (Kalimuthu et al., 2010; Junqueira-Gonçalves et al., 2015). The pharmacological evaluations of the extracts from natural product sources have led to isolation of beneficial compounds and antibiotics from natural sources (Vertika et al., 2012). Thus it is important to explore different types of medicinal plants for their antioxidant and antimicrobial potential.

In current study, we have reported the results of antioxidant, antifungal and antibacterial activities of the various solvent extracts of *Heliotropium curassavicu*m which will help to orient future investigations towards the finding of new, potent and safe antioxidant and antibiotic drug candidates.

II. METHODS

A. Plant material

The whole plant of *H. curassavicu*m L. was collected from Shamshi Khel area of River Kurram, Bannu (Pakistan) in May 2015. The Plant was identified by Dr Faizan ullah, Department of Botany, University of Science and Technology Bannu (Herbarium Voucher No: HC-1).

B. Extraction and fractionation

The collected plant material (1Kg) was air dried in shade for one week. The dried material was ground to fine powder by using grinder and then extracted three times with 80% aq. MeOH (2 litters, each soaking was for three days). The extracted material was filtered and dried to get methanolic crude extract. The crude extract (65gm) was defatted by using hexane to get defatted MeOH extract (50gm) and hexane extract (15gm). 45gm of defatted MeOH extract in dry form was suspended in water which was further fractionated by using dichloromethane and ethylacetate to get water (18gm),
dichloromethane (10gm) and ethylacetate (12gm) fractions. All the fractions were subjected to biological potential determination.

C. Phytochemical analysis

**Determination of total phenols**

The total phenolic content of the various fraction of *H. curassavicum* was determined by using standard method (Singleton and Rossi, 1965; Rajeswara et al., 2012). 0.005 gm of each fraction was dissolved in 1ml of the respective solvent to get sample fractions. 0.5 ml of each test sample fraction and 2.5 ml of 10-fold diluted Folin-Ciocalteu reagent were mixed with each other & then incubated at 32 °C for 5 minutes. 2 ml of a 7.5 % solution of sodium carbonate was added to the mixture. The resultant mixture was kept for 90 minutes in dark. The absorbance was measured at room temperature by spectrophotometer at 765 nm after 90 min. The total phenolic contents were calculated in terms of gallic acid equivalent (mg gallic acid equivalent/gm dry extract weight) by using Folin–Ciocalteu reagent.

**DPPH free radical scavenging assay**

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay of the various fractions of *H. curassavicum* was carried out by standard procedure (Brand-Williams et al., 1995). A DPPH solution was prepared by dissolving 3 mg of DPPH in 100ml of MeOH and kept in dark at room temperature until required.

The DPPH solution obtained was further diluted with methanol in order to get the working solution of correct optical density / absorption of 0.980 ±0.02 at 517 nm using Hitachi’s U-510 Tokyo Japan spectrophotometer. Stock solution stock solution of the various extracts of *Heliotropium curassavicum* were prepared in methanol to achieve the concentration of 2 mg/10ml. Dilutions were made to obtain concentrations of 1mg/10ml, 0.5mg/10ml and 0.25mg/10ml. The stock and diluted solution (100 μl each) were mixed in glass tubes with 1 ml of methanolic sol of DPPH. The glass tubes were then shaken well and were kept at room temperature for 30 minutes in dark. Ascorbic acid was used as standard. The absorbance was then recorded at 517 nm via Hitachi’s U-5100 Tokyo Japan spectrophotometer. The DPPH antioxidant activity was analyzed as percentage scavenging effect, as given by the equation:

Scavenging effect (%) = [(absorbance of control- absorbance of test sample/absorbance of control) ] ×100

**The ABTS⁺ cation scavenging assay**

The ABTS⁺ cation radical scavenging assay was carried out for analyzing the antioxidant activity of the various fractions of the *H. curassavicum*. The stock solution of the various extracts of *H. curassavicum* was prepared in methanol to achieve the concentration of 2 mg/10ml & then dilution was made to obtain concentrations of 1mg/10ml, 0.5mg/10ml and 0.25 mg/10ml. ABTS stock solution was prepared by mixing 2.45 mM aqueous solution of potassium persulfate with 7 mM aqueous solution of ABTS radical cation in dark and was incubated for 24 hours. The ABTS⁺ cation solution was further diluted at 30°C to get the absorbance of 0.7±0.005 at 734 nm. The antioxidant activity (ABTS) of each fraction was determined by mixing 300 μl of the test sample with 3000 μl of working standard in microcuvette and was incubated for 6 minutes. The decrease in absorbance was then calculated relative to the reaction control.

**Anti-fungal assay**

The anti-fungal assay of the various fractions of *H. curassavicum* was carried out by agar tube dilution procedure, by proceeding via the protocol as reported by Durai pandiyan and Ignacimuthu (2009). For the preparation of inoculums SDA media (Sabouraud dextrose agar) was used in order to grow fungus. 6.5 gm of Sabouraud dextrose agar was dissolved per 100 ml of distilled water in a conical flask and then autoclaved at 121 ºC for 21 min. The stock solution for each of the sample fraction was prepared by dissolving 12 mg per 1 ml DMSO. The antifungal agent Terbinafine solution 12 mg /ml in DMSO was kept as +ve control and pure DMSO as -ve control. The autoclaved medium is allowed to cool to about 50 ºC and then 4 ml of the non solidified medium loaded with 67 μl of the respective sample pipetted from the stock sol were poured into the tubes in duplicate for each species of fungus. Tubes were then placed in slanting position for 24 hrs for solidification. Each sample tube was inoculated with a piece of inoculums (4 mm diameter), taken from 7 days old culture of three fungal strains (Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus). Tubes with that negative and positive control i.e pure DMSO & Terbinafine were also inoculated and all the tubes were placed in incubator at 30 ºC for seven days. During incubation cultures were examined two times. The % inhibition was then measured by the following relation:

\[
\text{Fungal growth % inhibition} = \frac{[(100 - \text{linear growth in test sample in mm}) / \text{linear growth in control in mm})] \times 100}
\]

**Determination of antibacterial activity**

**Biological Materials**

Test organisms, e.g. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Escherichia coli* were used.

**Biological Test**

Antibacterial activity of the various solvent extracts of *H. curassavicum* was carried out by Agar diffusion assay (Leven et al., 1979) against four bacterial species. Out of four two organisms are Gram positive (*S. aureus* and *Staphylococcus aureus*).
& S. epidermidis) and two are Gram negative (K. pneumonia & E. coli). Sample fractions were prepared by dissolving 15 mg of each of the six fractions of H. curassavicum in 1 ml DMSO. 2.8 gm of the agar was dissolved in 100 ml of distilled water to prepare agar media. The media was then allowed to autoclave. Each Petri plate was loaded with 35 ml of the autoclaved media. The media was then allowed to autoclave. Each Petri plate was loaded with 35 ml of the autoclaved medium and then allowed to cool down to solidify. Six holes were cut in the periphery and one in the centre of the plate by using cork borer (sterile). The holes (cups) were insured to distribute properly. Plates were set in the plate by using cork borer. The holes were cut in the periphery and one in the centre of the plate. The plates were allowed for 2 hours at room temperature to ensure proper distribution of sample, and then kept face upward in incubator for 24 hours at 37 °C. After incubation, the diameters of the zones of inhibition were measured in mm (Leven et al., 1979; Kavanagh, 1963).

III. RESULTS AND DISCUSSION
The total phenolic content in various fractions of the H. curassavicum was found via Spectrophotometer. The highest value was shown by methanolic crude while lowest value by dichloromethane fraction.

One ranking of various fractions of H. curassavicum by phenolics content is as, methanolic crude > defatted methanolic > ethylacetate > water fraction > Hexane > dichloromethane. Phenolic compounds have been reported for their antibiotic and antioxidant properties (Junqueira-Gonçalves et al., 2015). Phenolic compounds quench the reactive oxygen species generated as a result of oxidative stress which cause toxic effects on biomolecules of cells (Hamama et al., 2017).

The DPPH antioxidant inhibition of the various fractions of H. curassavicum was found to be concentration dependent and decrease with decrease of concentration as shown in Table 2. The Dichloromethane fraction has the highest scavenging activity followed by Aqueous > Ethylacetate > Defatted methanolic > n-Hexane > Methanolic crude.

The antioxidant capacity of the various fractions of H. curassavicum via ABTS assay is shown below in Table 3. The Dichloromethane has highest scavenging activity followed by Ethylacetate > Aqueous > Methanolic crude > Defatted methanolic > n-Hexane.

Plant extracts exhibit antioxidant properties and are therefore rich sources of natural antioxidants (Popovic et al., 2006; Ciz et al., 2010). Previous studies of Wani et al. (2018) and Ahmad et al. (2014) have shown that aqueous and methanolic extract of Heliotropium indicum and Heliotropium baciferum exhibited antioxidant activity.
The antifungal assay % inhibition of the various extracts of *H. curassavicum* against three strains of fungi was measured as given below,

**A. flavus**: Higher antifungal activity against *A. flavus* was recorded for ethylacetate followed by defatted methanol > methanolic crude > dichloromethane > water > n-hexane

**A. niger**: Higher antifungal activity against *A. niger* was recorded for dichloromethane followed by defatted methanol > ethylacetate > methanolic crude > n-hexane > water fraction.

**A. fumigatus**: Higher antifungal activity against *A. fumigatus* was recorded for defatted methanol followed by n-hexane > methanolic crude > ethylacetate > dichloromethane > water fraction.

Fungi are causative agents of many diseases of both plants and animals. Results of current study revealed that various fractions of *H. curassavicum* such as defatted methanol, ethyl acetate and dichloromethane exhibited higher antifungal activity.

These antifungal activities are usually due to the presence of different kinds of biologically active compounds in plant extracts (Ammar et al., 2017).

All the six fractions of the plant showed significant antibacterial ability. The antibacterial activity (zones of inhibition) of the various solvent fractions of *H. curassavicum* against four strains of bacteria (two Gram positive & two Gram negative) was measured (Table 5) given below & discussed as,

**Klebsiella pneumonia** (Gram -ve): Ethylacetate fraction has the highest zone of inhibition followed by n-hexane > dichloromethane > methanolic crude > defatted methanolic > aeous.

**Escherichia coli** (Gram -ve): n-Hexane fraction has the highest zone of inhibition followed by methanolic crude > dichloromethane > defatted methanolic > ethylacetate > aqueous.

**Staphylococcus aureus** (Gram +ve): n-Hexane has the highest zone of inhibition followed by methanolic crude > ethylacetate > dichloromethane > defatted methanolic > aqueous.

**Staphylococcus epidermidis** (Gram +ve): Ethylacetate has the highest zone of inhibition followed by n-hexane > defatted methanolic > methanolic crude > dichloromethane > aqueous.

Similarly previous studies of Ahmad et al., (2015) have reported that n-hexane, ethylacetate and aqueous extracts of *Heliotropium bacciferum* exhibited antibacterial activity against the tested bacterial strains.

### IV. CONCLUSIONS

The selected plant was screened for total phenolic content, Antioxidant DPPH, Antioxidant ABTS, Antifungal activity and Antibacterial activity. Summary of the data obtained clarified that methanolic crude extract showed highest phenolic contents. Good antioxidant activity (both DPPH & ABTS) was showed by dichloromethane fraction of the plant. Significant antibacterial activity (zones of inhibitions) was measured against *K. pneumonia, E. coli, S. aureus and S. epidermidis*. All fractions of the plant were found to have significant antifungal activity against *A. flavus, A. niger, A. fumigatus*.

### REFERENCES


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**Table 5. Antibacterial activity of the various fractions of *H. curassavicum***

<table>
<thead>
<tr>
<th>Bacterial Strains</th>
<th>M/crude</th>
<th>Defatted Methanolic Extract</th>
<th>Hexane</th>
<th>Dichloromethane</th>
<th>E/acetate</th>
<th>Aqueous</th>
<th>Control</th>
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<tr>
<td><em>K. pneumonia</em></td>
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<td>10</td>
<td>11.5</td>
<td>11</td>
<td>13</td>
<td>9</td>
<td>16.5</td>
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<tr>
<td><em>E. coli</em></td>
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<td>14</td>
<td>17.5</td>
<td>14.5</td>
<td>12.5</td>
<td>9</td>
<td>24</td>
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<tr>
<td><em>S. aureus</em></td>
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<td>10.5</td>
<td>13.5</td>
<td>12</td>
<td>12.5</td>
<td>10</td>
<td>16.5</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>14.5</td>
<td>15</td>
<td>17</td>
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Received: December 22, 2017.

Sent to Subject editor: February 01, 2018.

Accepted: August 27, 2018.

Recommended by Subject Editor Maria L. Ferreira