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# Phytochemical analysis, antipyretic and antifungal activities of *Solanum nigrum* L.

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**Abstract**
Medicinal plants contain chemical compounds capable of preventing and fighting oxidative related diseases. In the present study phytochemical analysis of ethanolic, methanol, and chloroform extracts and anti-pyretic, antifungal activities in methanolic, ethanolic and chloroform extracts of *Cyrtomium caryotideum*, were carried out. Phytochemical analysis showing that ethanolic and methonalic extract contained carbohydrates, flavonoids, alkaloids, phlobatannins, saponins, tannins, phenols, terpenoids and cardiac glycosides, while alkaloids, phlobatannins, glycosides and protein were absent. Quantative phytochemistry showed the presence of flavonoids in chloroform extract as (14.20±0.15 mg/ml), while alkaloids as (12.10±0.15 mg/ml), phenolic as (10.45±0.10 mg/ml), Saponins (06.22±0.14 mg/ml) and Tannins as (04.60±0.65 mg/ml). Pharmacological activities such as, Anti-pyretic activity were carried out by brewer yeast induced pyrexia. Dose of 600 mg/kg of extract showed significant anti-pyretic activity i.e. (59.43%) when compared with positive control paracetamol i.e. (37.24 °C) inhibition (73.23%). Antifungal activity showing the most active among the extracts range from (17.00±0.48 mm) zone of inhibition at the concentration of18 mg/µl against *Verticillium* followed by *Pythium* range from (16.27±0.93 mm), *Acremonium* (16.20±1.89 mm) and *Trichoderma* (16.11±0.82) with concentration of 12 mg/µl.

**Keywords:** Phytochemical analysis, antipyretic, antifungal activities, *Solanum nigrum* L.

## 1. Introduction
Phytochemicals are biological active and naturally occurring chemical compounds found in plants, it have medicinal values for human benefits (Hasler & Blumberg, 1999) [13]. Phytochemicals protect plants from damage, diseases and contributed to the plants color, flavor and odor. The plant chemicals that protect plant cells from environmental extortions such as stress, drought, pollution, pathogenic attack (Gibson *et al*., 1998) [10]. Plants are contain various phytochemical constituents such as tannins, flavonoids terpenoids, phenolic acids, vitamins, lignins, quinines, stilbenes, amines, coumarins, betalains, alkaloids, and other phytochemicals, which have the potential of antioxidant activity (Zheng and wang, 2001) [34]. Research have showed that several of these antioxidant compounds have ant atherosclerotic, anti-inflammatory, anti-mutagenic, antitumor, antibacterial, antiviral and anti-carcinogenic activities (Ganesan *et al*., 2017) [9]. In modern research, plant phytochemicals, formerly with unknown and biological activities, have been widely studied as a basis of medicinal agents (Krishnaraju *et al*., 2015) [34]. Therefore, it is estimated that plants phytochemicals with sufficient antibacterial ability will be used for the cure of bacterial infections (Gracelin *et al*., 2013) [11]. Pyretic is defined as the elevation of core body temperature above the in normal adults, the average oral temperature is 36.98C (98.58F). In oncology practice, a single temperature of more than 38.3°C (101°F) or three readings (at least 1 hour apart) of more than 38 °C (100.4°F) are considered significant. Lower temperature elevations in the very young or old and in patients receiving steroids or other immune suppressants are considered abnormal (Mackowiak *et al*., 1997) [21]. Ferns and their allies are in a major division of the plant Kingdom called Pteridophyta and they have been around for millions of years. There are over 250 different genera and 12,000 species of ferns reported all over the world (Chang *et al*., 2011) [5]. It has been observed that pteridophytes are not infected by microbial pathogens which may be one of the important factors for the evolutionary success of pteridophytes and the fact that they survived for more than 350 million years (Sharma and Vyas 1985) [29]. *Solanum nigrum* L. is a member of family Dryopteridaceae. It is a most common terrestrial herb growing in semi-shaded localities Ghats at lower altitudes and completely dry soils in plains areas.

Their rhizome erect, densely covered with lanceolate, blackish brown scales, stipe stramineous, i.e. 16-32 cm to 2-3 mm in diameter with a central blackish brown stripe. Lamina is oblong or oblong-lanceolate and whole plant used as anti-bacterial and anthelminthic purposes (Naqshi, 2016) [22].

## 2. Methodology

### 2.1 Sampling and Identification

In the present study, *Solanum nigrum* L. whole plant was collected in November, 2020 from Mansehra Hazara division of Khyber Pakhtunkhwa Province Pakistan. With the help of Flora of Pakistan plant were taxonomically identified and placed in the Herbarium of Abdul Wali Khan University Mardan Garden campus.

### 2.2 Solvents

For the crude extract preparation of the *Solanum nigrum* L. methanol, ethanol and chloroform was used.

### 2.3 Crashing and filtration of the plant

The dried plant was crushed with the help of electric grinder. Powder were kept in air tight plastic bottles for further phytochemical, nutritional analysis, pharmacological and antifungal activities. 15 gm of plant powdered was retained in distinct conical flask and 90 ml of solvent i.e. (methanol, ethanol and chloroform) was added to the powdered separately. With the help of aluminum foil the flask were covered and retained in shaker for 72 hrs for the shaking purposes. After 72 hrs the extracts were filtered with the help of Whatman filter paper and then through filtration process plant extracts were removed (Pirzada *et al*., 2010) [25].

### 2.4 Phytochemical analysis

**Qualitative study**

The plant extract i.e. methanol, ethanol and chloroform were tasted for the absence or presence of phytochemical constituents' like alkaloids, tannins, Phlobatannins, flavonoids, carbohydrates, phenols, saponin, cardiac glycosides, proteins, glycosides and terpenoids (Soni *et al*., 2011) [31].

#### 2.4.1 Tests for Alkaloids

For detection of alkaloids, a few drops of Wagner's reagent (Potassium iodine) are add to 2 ml of all three methanol, ethanol and chloroform extracts. The presence of alkaloids were checked by the formation of reddish brown precipitate (Khandewal *et al*., 2015) [18].

#### 2.4.2 Tests for Tannins

For the detection of tannins Ferric chloride test was done. Ferric chloride ($FeCl_3$) solution was mixed with all three extracts separately. Formation of blue green coloration showed the presence of tannins. (Kokate *et al*., 2008) [19].

#### 2.4.3 Tests for Phlobatannins

In test tubes 0.5 ml of all the three extracts was taken separately, added 3ml distilled water and shaken for a few minutes then 1% aqueous hydro chloride (HCl) was added and boiled on water both. Presence of phlobatannins is confirmed by the formation of red color (Wadood *et al*., 2013) [32].

**2.4.4 Tests for Flavonoids:** For flavonoids detection, sodium hydroxide (NaOH) solution was added to all the three extracts of the plant. Red precipitation formation of indicate the presence of flavonoids (Kokate *et al*., 2008) [19].

#### 2.4.5 Tests for Carbohydrates

For detection of carbohydrates, 0.5 ml of all three extracts were treated with 0.5 ml of Benedict's regent. Solution were heated for 2 minutes on a water bath. Reddish brown precipitate indicates the presence of carbohydrate (Bussau, *et al*., 2002) [4].

#### 2.4.6 Tests for Phenols

For phenol detection, 2 ml of ferric chloride ($FeCl_3$) solution were added to 2 ml of all the three extracts separately in a test tube. Deep bluish green coloration formations showed the presence of phenol (Dahiru *et al*., 2006) [7].

#### 2.4.7 Tests for Saponins

For the detection of saponin, in test tube 5 ml of all three extracts were shaken vigorously. The presence of saponins was confirmed by froth formation (Rajesh *et al*., 2016) [26].

#### 2.4.8 Tests for (Cardiac) Glycosides

For cardiac glycosides detection, 2 ml of all three extracts solution were shaken with 2 ml of glacial acetic acid than added few drops of concentrated sulphuric acid ($H_2SO4$) and iron tri chloride ($FeCl_3$). Brown ring formation indicated the presence of Cardiac glycosides (Soni *et al*., 2011) [31].

#### 2.4.9 Tests for proteins

**Xanthoproteic test:** For the detection of protein, 1 ml from of all three extracts were treated with 1ml of concentrated nitric acid ($HNO_3$) solution. The presence of proteins indicated by the formation of yellow color (Rajesh *et al*., 2016) [26].

#### 2.4.10 Tests for terpenoids

**Salkowski test:** One ml of plant extracts (methanol, ethanol and chloroform) were added with 2 ml of chloroform. After that carefully added concentrated sulphuric acid ($H_2SO_4$) along the sides of tube to form a layer. The reddish brown coloration formation showed the presence of terpenoids (Dahiru *et al*., 2006) [7].

#### 2.4.11 Tests for Glycosides

For the detection of glycosides, 5% of Ferric chloride solution and 1 ml glacial acetic acid were added to 5 ml of all three extracts and then further addition of few drops of concentered sulphuric acid ($H_2SO_4$). The presence of glycosides was conformed through the formation of greenish blue color (Rajesh *et al*., 2016) [26].

### 2.5 Quantitative phytochemicals analysis

#### 2.5.1 Determination of total flavonoids constituents

Ethanol, methanol and chloroform extracts were used for the detection of total flavonoids contents. Total flavonoids quantification were done by taking 0.5 g of plant extracts. Than the sample were mixed with 4.3 ml methanol and then more addition of 0.1 ml of aluminum tri chloride from 10% prepared solutions of aluminum tri chloride laterally. Potassium acetate (0.1 ml) were added upto total volume 5 ml. The mixtures were shaken by vortex to make uniform solution and then mixture were placed at room temperature for 30 minutes for incubation. After incubation process, the

absorption were checked at 415 nm in spectrum. Quercetin were used as a standard (Daffodil *et al*., 2013) [6].

**2.5.2 Determination of Total Phenolic constituents**

Total phenolic quantification done by the addition of 0.5 g plant extract to 1 ml of 80% ethanol. Then the mixture were centrifuged for 15 minutes at 12,000 rpm. After that the supernatant were kept in test tube and these process were repeated 6 times. After collecting the supernatant were placed in water bath for drying. Distilled water was added to the supernatant until its volume reached to 3 ml. 2 ml ($Na_2CO_3$) of 20% were added in solution. To this 0.5 ml Folin-ciocalteau regent were added. After 5 minutes more addition of 2 ml ($Na_2Co_3$) from 20% $Na_2Co_3$ in this solution. Solution were mixed homogenously and then the test tube were brought in to the water bath in boiling water. At 650 nm their absorbance were checked. The Catechol was used as a standard (Grcelin *et al*., 2013) [11].

**2.5.3 Quantification of total alkaloids constituents**

5 gm of the all the three extracts were balanced in a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered than allowed for 4 hours to stand. Extracts was filtered and was concentrated on a water bath to one-quarter of the original volume. Until the precipitation was complete Drop wise to the extract concentrated ammonium hydroxide was added. The solution was allowed to settle and collected the precipitated and washed with dilute ammonium hydroxide and then filtered. The residue alkaloid, were dried and weighed (Gracelin *et al*., 2013) [11].

**2.5.4 Determination of total tannins constituents**

Sample having 500 mg were weighed in a 50 ml plastic bottle. Add 50 ml of distilled water and shake for 1 hour in a mechanical shaker. 50 ml volumetric flask was filtered and made up to the mark. Into a test tube, 5 ml of the filtered was pipetted out and mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium Ferro cyanide. At 120 nm their absorbance was cheeked out (Gracelin *et al*., 2013) [11].

**2.5.5 Determination of total saponins constituents**

Into a conical flask 20 gm of each extracts were put and 100 cm3 of 20% ethanol, aqueous were added. Kept in water bath for 4 hours the samples were heated with constant stirring at about 55 °C. Residue were re-extracted with another 200 ml 20% ethanol, and mixture were filtered. At about 90 °C through water bath the combined extracts were reduced to 40 ml. Into a 250 ml separatory funnel the concentrate was transferred and 20 ml of diethyl ether was added and vigorously shaken. 60 ml of n-butanol was added. With 10 ml of 5% aqueous sodium chloride the combined n-butanol extracts were washed twice. In a water bath the remaining solution was heated. Samples were dried in the oven to a constant weight after evaporation; the saponin content was calculated (Gracelin *et al*., 2013) [11].

**3. Pharmacological activities**

Pharmacological activities was carried out in methanolic extracts of rhizome of *Solanum nigrum* L. plant.

**3.1 Experimental animals**

Both sexes of the albino mice of weight about 25 - 30 gm were brought from National Institute of Health Islamabad. Animals were supplied with adlibitum water and standard pellet diet.

**3.2 Drugs used and chemicals used**

Aspirin (Bayer, Germany), Paracetamol (Glaxo Smith Kline, U.K), were used as standard drugs in the experiment for selected activities. Methanol 95% (Merck, Germany), Normal saline (Immunasol NS, A.Z. Pharmaceuticals Co.pak), Brewer's yeast.

**3.3 Evaluation of Antipyretic activity by brewer's yeast induced pyrexia**

For evaluation of antipyretic activity five groups of albino mice were taken. The initial temperature of all mice were checked. For antipyretic activity, the brewer yeast solution was prepared by dissolving 7% brewer yeast in 100 ml water. The paracetamol solution was used as a standard. Paracetamol solution was injected to the mice of group 2nd. To the group 3rd, 4th and 5th methanolic extract at the dose of 200, 400 and 600 mg/Kg was injected. After the injection of standard drug (paracetamol) and methanolic extract, the temperature of all the mice were checked at interval of one hour up to four hours.

The results of methanolic extract was compared with the standard drug (paracetamol) for low or high antipyretic potential. % reduction in temperature are calculated by using the following formula (Janaranjani *et al*., 2014) [35].

$$\% \text{ reduction} = B - Cn/B - A \times 100$$

B= temperature after pyrexia induction;
Cn= temperature after 1, 2, 3, 4 and 5 hours, A=temperature of normal body

**4. Anti-fungal activity**

Antifungal activity was carried out in ethanolic, methanolic and chloroform extracts.

**4.1 Media preparation**

Dissolve 39 gm of Potato dextrose agar (PDA) in 1 litre of distilled water, sterilized by autoclaved at 15psi (121 °C) for 15 minutes. Cool to room temperature and pour into sterilized Petri plates to solidify. Kept at room temperature to solidify for 30 minutes.

**4.2 Agar well diffusion method**

The micropipette using, in sterile distilled water (SDW) was placed of 100µl of different fungal cultures over the surface of an agar plate and with the help of a sterile inoculation loop it was spread, hole were made in each of the culture plates using a sterile cork borer. 75 µl of crude extract of selected plants were added. Culture plates incubated at 37 °C, and after 24 hours the results were detected depending on the fungal growth. Around each well clear zone was measured in mm, and clear zone formation exposed the antifungal activity of all extract against the each fungus. All activity were for formed in triplicated and then calculated the standard deviation. Agar well diffusion method was followed as described by Samie *et al*., (2010) [28].

**5. Statistical analysis**

Individual triplicate experiment were performed for all tests. Data are shown as mean±standard error of mean (S.E.M., *n* = number of Experiments). Statistical studies were obtained

by the one way analysis of variance (ANOVA), followed by the Dennett's test where necessary. $p<0.05$ was considered Significant.

## 6. Results and Discussion

### 6.1 Phytochemical analysis

In the present research study phytochemical analysis of methanolic, ethanolic and chloroform extracts of *Solanum nigrum* L. and their pharmacological activities of methanolic extracts anti-pyretic were carried out. Also antifungal activities in ethanolic methanolic and chloroform extracts were studied.

### 6.2 Phytochemical detection in the leaves and rhizome of *Solanum nigrum* L.

Qualitative analysis of *Solanum nigrum* L. was carried out for the detection of alkaloid, flavonoids, carbohydrate, phlobatannins, glycosides, saponins, phenol, terpenoids, tannins, cardiac glycosides and proteins. The results showed that alkaloids, flavonoids, carbohydrates, phlobatannins, saponins, phenols, terpenoids, tannins, cardiac glycosides was found in methanolic and ethanolic extracts, while alkaloids, phlobatannins, glycosides and protein were absent in the chloroform extracts. Flavonoids, carbohydrates, saponins, phenols and terpenoids were found present in in the rhizome methanolic and ethanolic extracts. In these results +++ indicate that the secondary metabolites present in highest amount, the ++ indicated that the moderate level of phytochemicals' are present and the + indicated that low level of phytochemicals are present and – indicated that the phytochemicals are absent in all these three extracts plants (Table 1, 2).

**Table 1:** Qualitative phytochemicals detection in the rhizome of *Solanum nigrum* L. in methanolic, ethanolic and chloroform extracts

| S. No | Phytochemical test | Methanolic | Ethanolic | chloroform |
|---|---|---|---|---|
| 1 | Alkaloid | +++ | ++ | _ |
| 2 | Flavonoids | + | +++ | + |
| 3 | Carbohydrate | +++ | + | + |
| 4 | Phlobatannins | +++ | ++ | _ |
| 5 | Glycosides | + | +++ | + |
| 6 | Saponins | + | ++ | + |
| 7 | Phenol | +++ | ++ | + |
| 8 | Terpenoids | ++ | +++ | _ |
| 9 | Tannins | +++ | ++ | + |
| 10 | Cardiac glycosides | ++ | + | _ |
| 11 | Proteins | ++ | + | _ |

**Key:** +++: present highest level, ++ showed moderate level, + showed low level - absent

**Table 2:** Phytochemicals detection in the leaves of *Solanum nigrum* L. in methanolic, ethanolic and chloroform extracts

| S. No | Phytochemical test | Methanolic | Ethanolic | chloroform |
|---|---|---|---|---|
| 1 | Alkaloid | ++ | + | _ |
| 2 | Flavonoids | + | +++ | + |
| 3 | Carbohydrate | +++ | ++ | + |
| 4 | Phlobatannins | +++ | ++ | _ |
| 5 | Glycosides | ++ | +++ | _ |
| 6 | Saponins | +++ | +++ | + |
| 7 | Phenol | +++ | + | + |
| 8 | Terpenoids | ++ | +++ | + |
| 9 | Tannins | +++ | ++ | + |
| 10 | Cardiac glycosides | ++ | +++ | _ |
| 11 | Proteins | ++ | ++ | _ |

**Key:** +++: present highest level, ++ showed moderate level, + showed low level - absent

### 6.3 Total Phenolic, Flavonoids, Tannins, Saponins and Alkaloids Contents in chloroform, methanol and ethanol

Highest amount of flavonoids was found in the chloroform extract as (14.20±0.15 mg/ml) followed by Alkaloids (10.14±0.12 mg/ml), phenolics (10.45±0.10 mg/ml), Saponins (06.22±0.14 mg/ml) and lowest amount of Tannins was found in (04.60±0.65 mg/ml). The flavonoids was found in highest amount in methanolic as (17.55±0.10 mg/ml), followed by phenols (13.25±0.50 mg/ml), Tannins (11.55±0.30 mg/ml), Alkaloids (10.05±0.10 mg/ml) and Saponins was found in lowest amount (08.40±0.45 mg/ml). The flavonoids was found in highest amount in methanolic as (13.25±0.50 mg/ml), followed by phenols (12.64±0.14 mg/ml), Alkaloids (09.50±0.15 mg/ml), Tannins (06.25±0.40 mg/ml) and Saponins was found in lowest amount (05.40±0.25 mg/ml). The data is showed in (Table 3, 4 and 5).

**Table 3:** Qualitative Phytochemicals detection of *Solanum nigrum* L. in chloroform extracts

| S. No. | Phytochemicals name | Concentration mg/ml |
|---|---|---|
| 1 | Total phenolics | 10.45±0.10 |
| 2 | Total flavonoids | 14.20±0.15 |
| 4 | Total Tannins | 04.60±0.65 |
| 5 | Total Saponins | 06.22±0.14 |
| 6 | Total Alkaloids | 10.14±0.12 |

**Table 4:** Qualitative Phytochemicals detection of *Solanum nigrum* L. in methanolic extracts

| S. No. | Phytochemicals name | Concentration mg/ml |
|---|---|---|
| 1 | Total phenolics | 13.25±0.50 |
| 2 | Total flavonoids | 17.55±0.10 |
| 4 | Total Tannins | 11.55±0.30 |
| 5 | Total Saponins | 08.40±0.45 |
| 6 | Total Alkaloids | 10.05±0.10 |

**Table 5:** Qualitative Phytochemicals detection of *Solanum nigrum* L. in ethanolic extracts

| S. No. | Phytochemicals name | Concentration mg/ml |
|---|---|---|
| 1 | Total phenolics | 12.64±0.14 |
| 2 | Total flavonoids | 13.25±0.50 |
| 4 | Total Tannins | 06.25±0.40 |
| 5 | Total Saponins | 05.40±0.25 |
| 6 | Total Alkaloids | 09.50±0.15 |

### 6.4 Pharmacological activities

For pharmacological activities methanolic extracts was used.

#### 6.4.1 Anti-pyretic activity

Anti-pyretic activity of *Solanum nigrum* L. whole plant was performed using brewer yeast induced pyrexia test. In experimental mice subcutaneous administration injection of yeast suspension markedly elevate the rectal temperature after 24 hours. Treatment with the *Solanum nigrum* L. extract at the doses of 200, 400 and 600 mg/kg decreased the rectal temperature at 3hours were 37.10±0.9 °C, 37.09±1.43 °C and 37.24±1.12 °C respectively. There was a dose dependent responses were observed in experimental mice. The antipyretic effect started as from the first hour and the effect was maintained for 3 hrs, after administration of the extract. Dose of 600 mg/kg of extract revealed significant anti-pyretic activity i.e. (46.12%) when it compared with positive control i.e. paracetamol (37.24 °C) inhibition (61.53%). The data was showed in table (6).

**Table 6:** Anti-pyretic activity of *Solanum nigrum* L. whole plant in methanolic extracts

| Rectal temperature (°C) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Drug** | **Dose** | **Before Yeast injection (Mean ± SEM)** | **After Yeast injection (Mean ± SEM)** | **1 hour (Mean ± SEM)** | **2 hours (Mean ± SEM)** | **3 hours (Mean ± SEM)** | **% Anti-pyretic Inhibition** |
| Control | N/S | 37.64±0.26 | 38.93±0.01 | 38.19±0.25 | 37.87±0.23 | 37.59±0.15 | …….. |
| Paracetamol | 150 mg/Kg | 37.10±0.25 | 38.94±0.19 | 38.72±0.10 | 37.67±0.15 | 37.74±.03 | 73.23% |
| Methanolic extract | 200 mg/Kg | 37.07±0.21 | 38.66±1.0 | 38.41±0.26 | 37.61±0.22 | 37.10±0.9 | 27.34% |
| Methanolic extract | 400 mg/Kg | 37.01±0.15 | 38.52±0.10 | 38.06±0.31 | 37.33±1.20 | 37.09±1.43 | 42.18% |
| Methanolic extract | 600 mg/Kg | 37.07±0.15 | 38.63±0.72 | 38.68±0.18 | 37.53±0.86 | 37.24±1.12 | 59.43% |

### 6.5 Antifungal activity of methanolic, ethanolic and chloroform extracts of *Solanum nigrum* L. against selected fungal strains

The results of antifungal activity showed that Crude methanolic Extract were active against all fungal species and showed different range of zone of inhibition. The extracts that is most active as (17.00±0.48 mm) zone of inhibition at the concentration of18 mg/µl against *Verticillium*, followed by *Pythium* (16.27±0.93 mm), *Acremonium* (16.20±1.89 mm) and *Trichoderma* (16.11±0.82) concentration of 12 mg/µl. And the ethanolic extracts showed maximum zone of inhibition (16.06±0.97 mm) at the concentration 12 mg/µl against *Trichoderma* fallowed by *Acremonium* (15.33±1.12 mm) at the concentration of 6 mg/µl and lowest amount of inhibition was showed against *Verticillium* (11.13±1.65 mm) and *Trichoderma* (10.062±1.32 mm). The chloroform extracts showed maximum inhibition against *Verticillium* (16.63±0.65 mm) at the concentration of 12 mg/µl, followed by *Trichoderma* (16.18±1.43mm) at the concentration of 6 mg/µl, *Alternaria* (14.40±1.34) at the concentration of 12 mg/µl and lowest amount of inhibition was showed *Acremonium* (10.23±0.88mm), *Pythium* (10.00±3.60 mm) and *Alternaria* (8.56±1.75 mm) at the concentration of 18 mg/µl and 6 mg/µl. The data are shown in table (7, 8 and 9).

**Table 7:** Antifungal activity of chloroform extracts of *Solanum nigrum* L. against selected fungal strains

| Extracts concentration | *Alternaria* | *Acremonium* | *Verticillium* | *Pythium* | *Trichoderma* |
|---|---|---|---|---|---|
| 6 mg/µl | 8.56±1.75 | 13.37±1.52 | 9.83±0.46 | 12.33±0.76 | 16.18±1.43 |
| 12 mg/µl | 14.40±1.34 | 10.47±0.67 | 16.63±0.65 | 13.47±1.32 | 14.09±0.87 |
| 18 mg/µl | 11.00±1.20 | 10.23±0.88 | 12.37±1.86 | 10.00±3.60 | 12.00±2.08 |

**Table 8:** Antifungal activity of ethanolic extracts of *Solanum nigrum* L. against selected fungal strains

| Extracts concentration | *Alternaria* | *Acremonium* | *Verticillium* | *Pythium* | *Trichoderma* |
|---|---|---|---|---|---|
| 6 mg/µl | 9.76±1.65 | 15.33±1.12 | 7.93±0.66 | 10.23±0.96 | 13.12±1.03 |
| 12 mg/µl | 14.50±1.25 | 13.57±0.87 | 12.83±0.95 | 14.27±1.22 | 16.06±0.97 |
| 18 mg/µl | 6.32±1.00 | 12.13±0.78 | 11.13±1.65 | 13.16±0.57 | 10.062±1.32 |

**Table 9:** Antifungal activity of methanolic extracts of *Solanum nigrum* L. against selected fungal strains

| Extracts concentration | *Alternaria* | *Acremonium* | *Verticillium* | *Pythium* | *Trichoderma* |
|---|---|---|---|---|---|
| 6 mg/µl | 6.89±0.11 | 16.20±1.89 | 13.44±0.53 | 10.67±1.30 | 11.65±1.75 |
| 12 mg/µl | 10.33±0.88 | 14.12±0.192 | 17.00±0.48 | 16.27±0.93 | 16.11±0.82 |
| 18 mg/µl | 10.80±0.58 | 9.89±0.63 | 13.62±0.87 | 9.17±1.01 | 14.77±1.13 |

## 7. Discussion

In the present research work phytochemical analysis of methanolic, ethanolic and chloroform extracts of *Solanum nigrum* L. and Anti-pyretic and antifungal activities in methanolic, ethanolic and chloroform extracts were studied. Qualitative analysis of *Solanum nigrum* L. was carried out for the detection of alkaloid, flavonoids, carbohydrate, phlobatannins, glycosides, saponins, phenol, terpenoids, tannins, cardiac glycosides and proteins. Results revealed that alkaloids, flavonoids, carbohydrates, phlobatannins, saponins, phenols, terpenoids, tannins, cardiac glycosides was found in methanolic and ethanolic extracts, while alkaloids, phlobatannins, glycosides and protein were absent in aqueous extracts. Carbohydrates, flavonoids, saponins, phenols and terpenoids were present in the rhizome methanolic and ethanolic extracts. Highest amount of flavonoids was found in the chloroform extract as (14.20±0.15 mg/ml) followed by Alkaloids (10.14±0.12 mg/ml), phenolics (10.45±0.10 mg/ml), Saponins (06.22±0.14 mg/ml) and lowest amount of Tannins was found in (04.60±0.65 mg/ml). The flavonoids was found in highest amount in methanolic as (17.55±0.10mg/ml), followed by phenols (13.25±0.50 mg/ml), Tannins (11.55±0.30 mg/ml), Alkaloids (10.05±0.10 mg/ml) and Saponins was found in lowest amount (08.40±0.45 mg/ml).*Solanum nigrum* L. extract at the doses of 200, 400 and 600 mg/kg decreased the rectal temperature at 3hours were 37.10±0.9 °C, 37.09±1.43 °C and 37.24±1.12 °C respectively. The dose of 600 mg/kg of extract showed remarkable anti-pyretic activity i.e. (59.43%) when is compared with positive control i.e. paracetamol (37.24 °C) inhibition (73.23%). Extract of *Solanum nigrum* L. were active against all fungal species and showed different range of zone of inhibition. The most active among the extracts (17.00±0.48 mm) zone of inhibition at the concentration of18 mg/µl against *Verticillium*. Followed by *Pythium* (16.27±0.93 mm), *Acremonium* (16.20±1.89 mm) and *Trichoderma* (16.11±0.82) with concentration of 12 mg/µl. And the ethanolic extracts showed maximum zone of inhibition (16.06±0.97 mm) at the concentration 12 mg/µl

against *Trichoderma* fallowed by *Acremonium* (15.33±1.12 mm) at the concentration of 6 mg/µl and lowest amount of inhibition showed against *Verticillium* (11.13±1.65 mm) and *Trichoderma* i.e. (10.062±1.32 mm). Chloroform extracts indicated maximum inhibition against the *Verticillium i.e.* (16.63±0.65 mm) at the concentration of 12 mg/µl, followed by *Trichoderma* (16.18±1.43 mm) at concentration of 6 mg/µl, and *Alternaria* (14.40±1.34) at the concentration of 12 mg/µl. Phytochemical components present in plant samples are known to be biologically active compounds and they are responsible for diverse activities such as antioxidant, antimicrobial, anticancer, antifungal, and antidiabetic (Hossain & Nagooru, 2011) [14]. Wide variety of pharmacological activities showed by different phytochemicals, which may help in protection against chronic diseases. Tannins, flavonoids, saponins, glycosides, and amino acids have anti-inflammatory and hypoglycemic activities. Steroids and terpenoids shows central nervous system (CNS) activities and analgesic properties. Because of their antimicrobial activity saponins are involved in plant defense system (Ayoola *et al*., 2008) [2]. These phytochemicals showed antimicrobial activity through different mechanisms. Proline-rich protein tannins have been found to form irreversible complexes (Shimada, 2006) [30] resultant in the inhibition of cell protein synthesis. (Parekh and Chanda, 2007) [24] stated that tannins are known to react with proteins that deliver the typical tanning effect which is vital for the cure of ulcerated or inflamed tissues. Herbs that have tannins as their key components are astringent in nature and are used for treating intestinal disorders such as dysentery and diarrhea (Dharmananda, 2003) [8]. Tannins and their derivatives are phenolic compounds considered to be primary antioxidants or free radical scavengers (Barile *et al*., 2007) [3]. So these observations therefore sustenance the use of *Solanum nigrum* L. in herbal medication remedies, thus suggesting that *Solanum nigrum* L. has potential as source of significant bioactive molecules responsible for treatment and anticipation of cancer. Presence of tannins in *Solanum nigrum* L. supports the outdated medicinal use of this plant in the treatment of different disorders. Alkaloid was alternative phytochemicals constituent's that observed in the extract of *Pteris quadriaurita*. One of the best common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely considered for their potential use in the reduction and ending of human cancer cell lines (Nobori, *et al*., 1994) [23]. One of largest group of phytochemicals i.e. alkaloids in plants which have remarkable effects on humans, and led to the improvement of powerful pain killer medicines (Kam and Liew, 2002) [17]. (Just *et al*., 1998) [16] exposed the inhibitory effect of saponins on inflamed cells. Saponin was present in *Solanum nigrum* L. extracts and it has maintained the usefulness of this plant in managing inflammation. Flavonoids, alternative phytochemicals indicated a diverse range of pharmacological activities like anti-inflammatory, antimicrobial, analgesic, anti-angionic, cytostatic, antioxidant and anti-allergic properties (Hodek *et al*., 2002) [13]. Several reports are offered on flavonoid groups which showing high potential biological activities such as anti-inflammatory, antioxidant, antiallergic reactions (Thitilertdecha *et al*., 2008) [36]. In the crude extracts bioactive compounds such as tannins and flavonoids were present. Until now, these phytochemical compounds were encouraging the antimicrobial and antioxidants activities. By fractionation the amount of active constituents in the crude extracts might be dilute or enhanced their concentrations (Anyasor *et al*., 2010) [1].

## 8. Conclusion

The above results confirmed that *Solanum nigrum* L. has better anti-inflammatory analgesic and antipyretic activity. The pharmacological activity of the *Solanum nigrum* L. may be due to the presence of phytochemical constituents. Some of these compounds possess analgesic, anti-inflammatory, antipyretic and antifungal activities. Further studies involving the purification of the chemical constituents of the plant and investigation in the biochemical pathway may results in the development of a potent analgesic, anti-inflammatory, anti-pyretic and antifungal agent with low toxicity and better therapeutic index.

## 9. References


1. Anyasor GN, Ogunwenmo O, Oyelana OA, Akpofunure BE. Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of Costus afer Ker Gawl (Costaceae). African Journal of Biotechnology. 2010;9(31):4880-4884.
2. Ayoola GA, Lawore FM, Adelowotan T, Aibinu IE, Adenipekun E, Coker HAB, *et al*. Chemical analysis and antimicrobial activity of the essential oil of Syzigium aromaticum (clove). African Journal of Microbiology Research. 2008;2(7):162-166.
3. Barile L, Messina E, Giacomello A, Marbán E. Endogenous cardiac stem cells. Progress in cardiovascular diseases. 2007;50(1):31-48.
4. Bussau VA, Fairchild TJ, Rao A, Steele P, Fournier PA. Carbohydrate loading in human muscle: an improved 1 day protocol. European journal of applied physiology. 2002;87(3):290-295.
5. Chang HC, Gupta SK, Tsay HS. Studies on Folk Medicinal Fern: An Example of "Gu-Sui-Bu". In Working with Ferns. Springer New York; c2011. p. 285-304.
6. Daffodil ED, Raja Lakshmi K, Mohan VR. Antioxidant activity, total phenolics and flavonoids of Salicornia brachiata Roxb. Leaf extracts (Chenopodiaceae). World J of Pharmacy and Pharmaceutical Sciences. 2013;2(1):352-366.
7. Dahiru D, Onubiyi JA, Umaru HA. Phytochemical screening and antiulcerogenic effect of Moringa oleifera aqueous leaf extract. African Journal of Traditional, Complementary and Alternative medicines (AJTCAM). 2006;3(3):70-75.
8. Dharmananda S. Gallnuts and the uses of Tannins in Chinese Medicine. ITM; c2003.
9. Ganesan K, Xu B. A critical review on phytochemical profile and health promoting effects of mung bean (Vigna radiata). Food Science and Human Wellness; c2017.
10. Gibson LF. Bacteriocin activity and probiotic activity of Aeromonas media. Journal of applied microbiology; c1998, 85(S1).
11. Gracelin DHS, Britto A, Kumar BJRP. Qualitative and quantitative analysis of phytochemicals in five Pteris species. Int. J Pharm Pharma Sci. 2013;5:105-7.
12. Hasler CM, Blumberg JB. Introduction. The Journal of nutrition. 1999;129(3):756S-757S.

13. Hodek P, Trefil P, Stiborová M. Flavonoids-potent and versatile biologically active compounds interacting with cytochromes P450. Chemico-biological interactions. 2002;139(1):1-21.
14. Hossain MA, Nagooru MR. Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medicinal plant *Cordyline terminalis* L. Kunth. Pharmacognosy Journal. 2011;3(24):25-30.
15. Huq S. Comparative phytochemical evaluation and biological activity screening of Murdannia nudiflora and *Tradescantia pallida*; c2015.
16. Just MJ, Recio MC, Giner RM, Cuéllar MJ, Máñez S, Bilia AR, *et al*. Anti-inflammatory activity of unusual lupane saponins from Bupleurum fruticescens. *Planta medica*. 1998;64(05):404-407.
17. Kam PCA, Liew S. Traditional Chinese herbal medicine and anaesthesia. Anaesthesia. 2002;57(11):1083-1089.
18. Khandelwal N, Kross EK, Engelberg RA, Coe NB, Long AC, Curtis JR. Estimating the effect of palliative care interventions and advance care planning on ICU utilization: A systematic review. Critical care medicine. 2015;43(5):1102.
19. Kokate A, Li X, Jasti B. HPLC detection of marker compounds during buccal permeation enhancement studies. Journal of pharmaceutical and biomedical analysis. 2008;47(1):190-194.
20. Lee-Thorp JA. On isotopes and old bones. Archaeometry. 2008;50(6):925-950.
21. Mackowiak PA, Borden EC, Goldblum SE, Has day JD, Munford RS, Nasr Away SA, *et al*. Concepts of fever: Recent advances and lingering dogma. Clinical Infectious Diseases. 1997;25(1):119-138.
22. Naqshi AR. Medicinal ferns of Kashmir, India. International Journal of Bioassays. 2016;5(7):1-9.
23. Nobori T, Miura K, Wu DJ, Lois A, Takabayashi K, Carson DA. Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature. 1994;368(6473):753.
24. Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. African Journal of Biomedical Research; c2007, 10(2).
25. Pirzada Z, Personick M, Biba M, Gong X, Zhou L, Schafer W, *et al*. Systematic evaluation of new chiral stationary phases for supercritical fluid chromatography using a standard racemate library. Journal of Chromatography A. 2010;1217(7):1134-1138.
26. Rajesh KD, Vasantha S, Panneerselvam A, Rajesh NV, Jeyathilakan N. Phytochemical analysis, *in vitro* antioxidant potential and gas chromatography mass spectrometry studies of Dicranopteris linearis. Asian J Pharm Clin Res. 2016;9(2):1-6.
27. Robert C, Thomas L, Bondarenko I, O'day S, Weber J, Garbe C, *et al*. Ipilimumab plus decarbonize for previously untreated metastatic melanoma. New England Journal of Medicine. 2011;364(26):2517-2526.
28. Samie A, Tambani T, Harshfield E, Green E, Ramalivhana JN, Bessong PO. Antifungal activities of selected Venda medicinal plants against Candida albicans, Candida krusei and Cryptococcus neoformans isolated from South African AIDS patients. African Journal of Biotechnology; c2010, 9(20).
29. Sharma BD, Vyas MS. Ethnobotanical studies on the ferns and fern allies of Rajasthan. Nelumbo. 1985;27(1-4):90-91.
30. Shimada T. Salivary proteins as a defense against dietary tannins. Journal of chemical ecology. 2006;32(6):1149-1163.
31. Soni J, Ansari U, Sharma D, Soni S. Predictive data mining for medical diagnosis: An overview of heart disease prediction. International Journal of Computer Applications. 2011;17(8):43-48.
32. Wadood A, Ghufran M, Jamal SB, Naeem M, Khan A, Ghaffar R. Phytochemical analysis of medicinal plants occurring in local area of Mardan. Biochem Anal Biochem; c2013.
33. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food chemistry. 2001;49(11):5165-5170.
34. Krishnaraju M, Kamatchi C, Jha AK, Devasena N, Vennila R, Sumathi G, *et al*. Complete sequencing of an IncX3 plasmid carrying blaNDM-5 allele reveals an early stage in the dissemination of the blaNDM gene. Indian journal of medical microbiology. 2015 Jan 1;33(1):30-8.
35. Janaranjani B, Prasanna G, Chitra M. Anti-inflammatory and Antipyretic Activities of Drynaria quercifolia Rhizome in Rats. Int. J Pharm. Sci. Rev. Res. 2014;13:57-61.
36. Thitilertdecha N, Teerawutgulrag A, Rakariyatham N. Antioxidant and antibacterial activities of *Nephelium lappaceum* L. extracts. LWT-Food Science and Technology. 2008 Dec 1;41(10):2029-35.