EVALUATION OF CYTOTOXIC, ANTIMICROBIAL AND ANTIINFLAMMATORY PROPERTIES FROM THE LATEX OF *IPOMEA STAPHYLINA*

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ABSTRACT

The plant *Ipomea staphylina* has been used in diverse traditional medication for the treatment of diseases and illness of human beings. The crude latex extract obtained from the stem of *Ipomea staphylina* was evaluated for cytotoxic, antimicrobial and wound healing properties. Cell viability and cytotoxicity assays such as Colony Formation method and Enzyme based methods that determined cell viability with a colorimetric method were performed to evaluate the medicinal properties of *Ipomea staphylina*. Similarly Microbiological Antibiotic Assay to determine the antimicrobial properties and wound healing properties were tested by determining the potent anti-inflammatory molecules that inhibited COX and LOX enzymes. Results showed that the latex crude extract of *Ipomea staphylina* showed potent Antimicrobial and Antiinflammatory properties, but the viability of the cells were unaffected.

INTRODUCTION

Inflammation is one of the major health problems. Inflammation refers to the health issues, including medical symptoms such as redness, pain and swelling of an affected organ or tissue (Kim et al. 2013a). Some cases like allergies, asthma, arthritis, and auto-immune disorders, certainly contain inflammatory components (Guerra et al. 2013; Hamsa et al. 2011). However, constant, low-level inflammation is now been connected with diseases like depression, heart disease, diabetes, Alzheimer’s, osteoporosis and cancer (Brouwers et al. 2013; Halaris, 2013; Nolan et al. 2013; Rücker, 2013; Dalamaga, 2014). Inflammation is also a key factor in skin aging. Many studies suggest that changes in gut micro biota, inflammation steroidal and non steroidal agents that relieve the pain and inflammation by blocking COX (cycooxygenase enzyme) and thus by stopping the production of prostaglandins, long term intake of such agents will result in acute gastrointestinal ulcers. Nature itself holds many anti-inflammatory substitutes around which have to be detected and explored (Lai et al. 2013; Kim et al. 2013b). This will reduce the risk and new potential components from plants have to be explored and detected that are safe and effective. *Ipomea staphylina* is one among such Species that is abundantly available. *Ipomea staphylina* belongs to the biggest family Convolvulaceae. Similarly bacterial resistance against antibiotic treatment has turned out to be a major threat to public health. Thus the need for new potent antimicrobial components has rapidly increased. Illustrations suggest the genus to be a source of therapeutic agents. Many of the *Ipomea sp.* has reported effective antimicrobial properties. *Ipomea genus* is thus extensively used in Traditional and Chinese medicine (Manvar et al. 2013). These species are used in diverse parts of the world for the healing of several diseases, such as, dysentery, diabetes, hypertension, arthritis, constipation, fatigue, rheumatism, inflammations, hydrocephaly, meningitis, kidney ailments and some of these species exposed potent hypoglycemic, antimicrobial, analgesic, spasmodic, hypotensive, anticoagulant, anti-inflammatory, psychotomimetic, spasmodenic, and anticancer activities. Literature suggests the presence of Polyphenols and Flavones that were reported. Polyphenols are natural source of antioxidants that human can consume as dietary supplements (Farias et al. 2013). They are widely used biologically to compensate and boost up the antioxidant requirements. Thus apart from the antioxidation supplement, it is extensively used as traditional remedies to heal human illness. Similarly flavones are also secondary metabolite that is of vast pharmacological importance both in vitro and in vivo. Presence of Flavonoids has shown potent antibacterial properties against many bacteria and as well as it is known to be cardio protective (Mignet et al. 2013). Thus we evaluated the antimicrobial, antiinflammatory and cytotoxic properties from the crude latex extract of *Ipomea staphylina* that is abundantly available.

MATERIAL AND METHODS

Preparation of Crude Latex extracts and estimation of Protein content

Known amount of Latex was collected from the twigs of *Ipomea staphylina* and mixed with little amount of water and centrifuged at 2500 rpm for 15 minutes at 4°C. Similar process was repeated with 50% ethanol and n-butanol. The Supernatant obtained was further used for the study.

Concentration of Protein present in the supernatant was estimated using BSA as standard by Lowery’s Method (Lowry et al. 1951).

Tests for Polyphenols and Flavones

Estimation of total polyphenols was carried out by Folic Ciocalcateu method using caffeic acid as standard and the similar experiment was carried out by Wosky and Salatino Colorimetric method to estimate flavones using Myricetin as standard (Boman et al. 1957). Further these results were confirmed by Concentrated Sulphuric acid test and Mg-HCl test that proved the presence of Bioflavonoids.

COX Activity Assay

The peroxidative activities of COX enzymes were calculated using COX activity kit where it contains a specific Chemiluminiscent substrate to detect the activity. 50µL of Tris-phenol Buffer and hematin solutions were added to all wells. COX preparations were added to all the wells except for Blank and Control. 25 µL of the latex extracts were added to all the wells and incubated for 2 hours. Microtiter plate was then checked for chemiluminiscent measurement. Later 50 µL of cold COX chemoluminescent substrate was added and the light output was measured in Relative Light Units (RU). To confirm and determine COX inhibition was determined and the s.d. values were reported.
Determination of Antimicrobial Activity

Bacterial plates were prepared using pour plate method. Antimicrobial Activity was tested using different concentrations of the sample. Staphylococcus aureus and E. coli cultures were used to determine the antimicrobial activity. Different concentrations ranging from 5µL to 1 ml plant extract was used and the height zone formation was noted for each clear zone formed. The samples were also tested using certain known concentrations of penicillin as well.

Determination of Cytotoxicity using MTT Assay

Cell Culture

J774.2 Cell line from mouse were cultured in Roswell Park Memorial Institute Medium (RPMI) containing 10% fetal bovine serum and 100 µL of penicillin and incubated at 37°C, 5% CO2 atmosphere (Gallet et al. 2009). Bacterial LPS (1 µg/ml) was added to stimulate the cells and fresh RPMI media was replaced. The cells were harvested and stored in 0.025% trypsin and 0.52mM phosphate buffer saline. These cells were used for MTT Assay.

MTT [3-(4,5-dimethyl thiazole-2-yl)-2,5-diphenyltetrazolium] Assay

MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. Cells were plated in PBS in 96-well (flat bottom). Cells of J774.2 Cell Line were exposed to different concentrations of plant extract, except for the control and incubated for 24h (Ryu et al. 2013). Then MTT reagent was added and incubated for 4 h. Post incubation a known amount of detergent solution (0.04N HCl+80% ethanol+20% TritonX) was added to all the test plates. The readings obtained were read at 570nm wavelength.

RESULTS AND DISCUSSION

Both the standard curves showed the presence of Bioflavonoid (figure 1 & 2). These were further confirmed by tests that showed pink to orange effervescence that proved the presence.

Graphical representation measuring COX activity

Bacterial free zones were clearly visible and the plant extracts of different concentrations showed potent antimicrobial activity that increased with increase in concentration of the sample (Alnajar et al. 2012) (figure 4). Results showed that the plant extract at 1 ml concentration inhibited more than the antibiotic penicillin (Table 1). The plant extract showed much potent results when compared to the antibiotics present. May be the bacteria has grown resistance towards the potential antibiotics. Thus development of potent natural components might turn in building up potential pharmaceuticals.

Table 1 Antimicrobial activity by Plant extract/Antibiotic

<table>
<thead>
<tr>
<th>Species</th>
<th>Concentration of plant extract</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>10µL</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>50 µL</td>
<td>++</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>100 µL</td>
<td>+++</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>300 µL</td>
<td>+++</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>700 µL</td>
<td>+++</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1 ml</td>
<td>+++</td>
</tr>
<tr>
<td>E. coli</td>
<td>10µL</td>
<td>++</td>
</tr>
<tr>
<td>E. coli</td>
<td>200 µL</td>
<td>+++</td>
</tr>
<tr>
<td>E. coli</td>
<td>300 µL</td>
<td>+++</td>
</tr>
<tr>
<td>E. coli</td>
<td>700 µL</td>
<td>+++</td>
</tr>
<tr>
<td>E. coli</td>
<td>1ml</td>
<td>+++</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>200 µL (Penicillin)-no plant extract was added</td>
<td>++</td>
</tr>
<tr>
<td>E. coli</td>
<td>200 µL (Penicillin)-no plant extract was added</td>
<td>++</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>500 µL (Penicillin)-no plant extract was added</td>
<td>+++</td>
</tr>
<tr>
<td>E. coli</td>
<td>500 µL (Penicillin)-no plant extract was added</td>
<td>+++</td>
</tr>
</tbody>
</table>

Legend: + denotes less inhibition; ++ - moderate inhibition; +++ - high inhibition; ++++ -very high inhibition
The sample did not affect the viability of the cells. The J774.2 Cells were unaffected by the cells and almost all the cell culture plates showed ~100% Cell viability. Thus the plant extract is not cytotoxic (figure 5).

**Figure 4** Clear zones were formed due to antimicrobial activity posed by the extracts of *I. staphyлина*

**Figure 5** Cell Viability assessments by MTT Assay

**CONCLUSION**

The increase in pervasiveness of multiple drug resistance has shown the importance and the need for development of new synthetic antioxidative, antibacterial, and anti-inflammatory drugs; however, the new drug is obligatory to search for new antioxidative, antimicrobial, and anti-inflammatory resource from alternative sources that are abundantly available at an affordable price. The exploit and exploration for drugs and nutritional supplements derived from plants have hastened in recent years. Ethno pharmacologists, botanists, microbiologists, and natural-products chemists are combing the phytochemicals that is being developed for treatment of infectious diseases. Whereas 25 to 50% of existing pharmaceuticals are derived from plants, none are used as antimicrobials. The study showed *Ipomoea staphyлина* posed potential therapeutic activities, however further studies on isolation and characterization of the molecules is required.

**Conflicts** None declared.

**REFERENCES**


