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

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ABSTRACT

The plant *Ipomoea 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 Antiinflamatory properties, but the viability of the cells were unaffected.

Keywords: *Ipomea staphylina*, antiinflammatory, antimicrobial, cell viability

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; Ridker, 2013; Dalamaga, 2014)**. Inflammation is also a key factor in skin aging. Many studies suggest that changes in gut micro biota, is also linked to inflammation. Thus many anti-inflammatory drugs are coming up in the industries that have numerable side effects **(Katz, 2013; Chimirri *et al.* 2013; Ren *et al.* 2013; *Ortuño Sahagún et al.* 2012)**. The treatment of inflammation steroidal and non steroidal agents that relieve the pain and inflammation by blocking COX (cyclooxygenase 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 Convulvulaceae.
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, spasmolitic, hypotensive, anticoagulant, anti-inflammatory, psychotomimetic, spasmogenic, and anticancer activities. Literature suggests the presence of Polyphenols and Flavones 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 cardioprotective **(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 Lowry'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 Woisky 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 Bioflavanoids.

COX Activity Assay

The perioxidative 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 chemiluminiscent substrate was added and the light output was measured in Relative Light Units (RLU).
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 Rosewell Park Memorial Institute Medium (RPMI) containing 10% fetal bovine serum and 100 µL of penicillin and incubated at 37℃, 5% CO_2 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,Sdiphenyltetrazolium bromide] Assay

MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-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 cell culture 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.

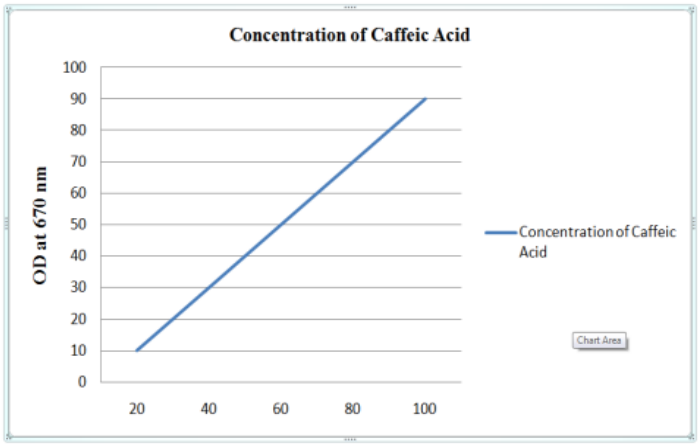

Figure 1 Estimation of Polyphenols

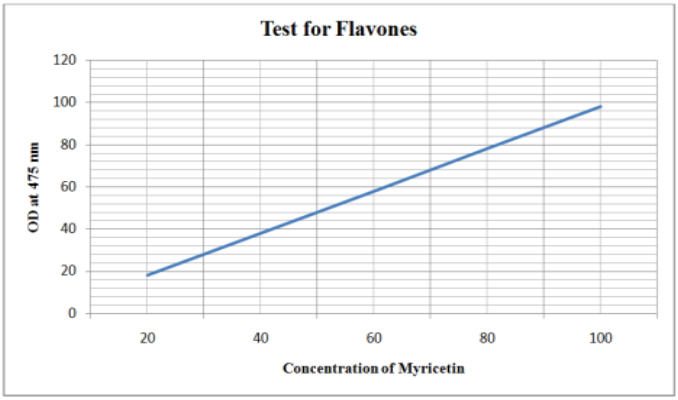

Figure 2 Estimation of Flavones

The plant extracts showed potent COX inhibition that clearly suggests the extracts contains potent anti-inflammatory components that could be of great therapeutic use (figure 3). The three different line show the activity inhibition by three different samples namely water, ethanol and n-butanol extracts respectively out of which ethanol extract posed the most significant inhibition activity **(Priyadarshini *et al.* 2013).** The problem of microbial resistance is rising and the point of view for the use of antimicrobial drugs in the future is still unsure. Therefore, measures must be taken to decrease this problem, for example, to manage the use of antibiotic, better research must be developed to understand the inherited mechanisms of resistance, and to bring out more studies to develop new drugs, that are of natural source (**Dutta *et al.* 2013; Tekwu *et al.* 2012)**.

Results showed the inhibition of COX studies where an exponential approach to stability was performed using the constants e^{-Ikt} - preincubation period, and $e^{-Ikt}{}_{opt}/‘1/‘1+K_m/s''$ - time to V_{opt}, where I - Inhibitor concentration, k - Second-order rate constant (k_{on}), t - preincubation time, t_{opt} - time to optimal velocity after the addition of substrate, K_m- Michaelis–Menten constant for substrate and s - substrate concentration. The second-order rate constant k and equilibrium K_i were concurrently solved from:

$$e\text{^}(-lktopt/(1/(1+Km/s))$$

The half life of the COX and the plant extract (inhibitor) was calculated using the formula:

$$F_0 = 1/(1+\left(\frac{K_i}{I}\right))$$

where K_i- Michaelis–Menten constant for calculated V_{max} and V_{opt} time

Standard deviations (S.D.) for the observed V_{max} ranged from 5 to 8% for the performed datasets.

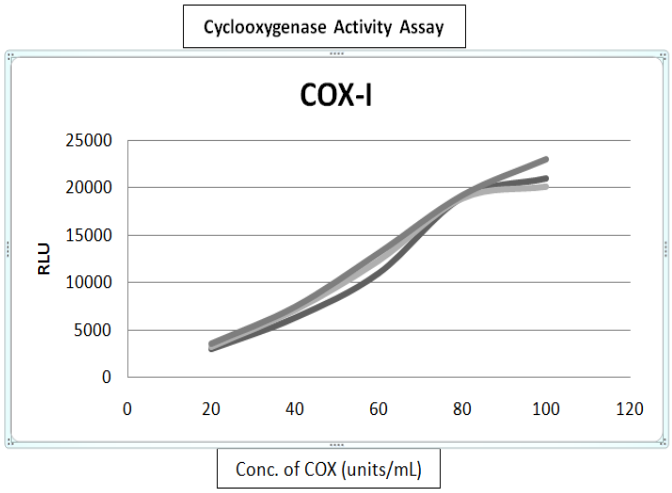

Figure 3 Graphical representation measuring COX activity

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 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

Species	**Concentration of plant extract**	**Inhibition**
Staphylococcus aureus	10µL	+
Staphylococcus aureus	50 µL	++
Staphylococcus aureus	100 µL	+++
Staphylococcus aureus	300 µL	+++
Staphylococcus aureus	700 µL	+++
Staphylococcus aureus	1 ml	++++
E. coli	10µL	++
E. coli	200 µL	+++
E. coli	300 µL	+++
E. coli	700 µL	++++
E. coli	1ml	++++
Staphylococcus aureus	200 µL (Penicillin)-no plant extract was added	++
E. coli	200 µL (Penicillin)- no plant extract was added	++
Staphylococcus aureus	500 µL (Penicillin)-no plant extract was added	+++
E. coli	500 µL (Penicillin)- no plant extract was added	+++

Legend: + denotes less inhibition; ++ - moderate inhibition; +++ - high inhibition; ++++ -very high inhibition

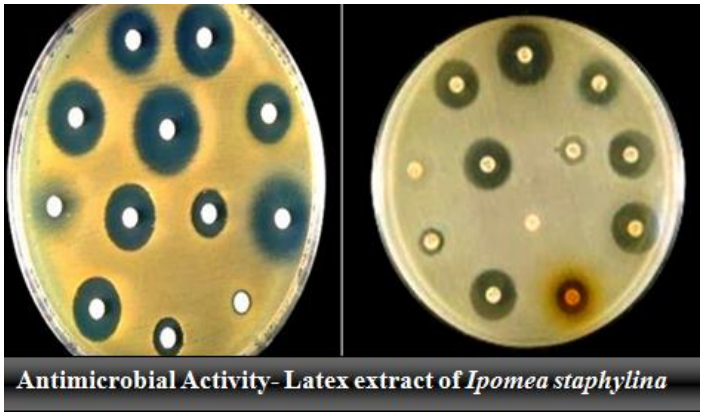

Figure 4 Clear zones were formed due to antimicrobial activity posed by the extracts of *I. staphylina*

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).

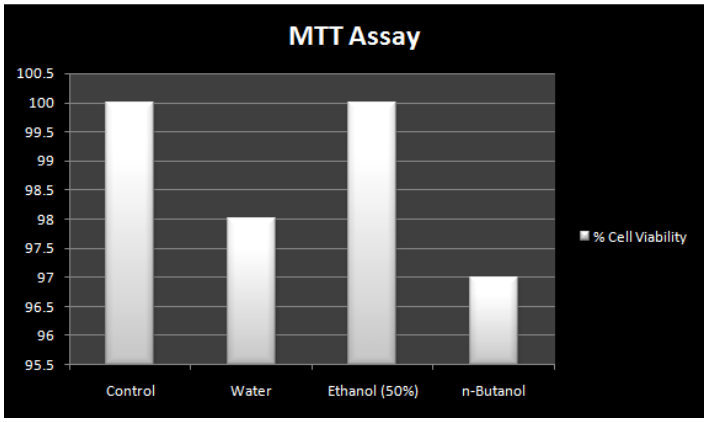

Figure 5 Cell Viability assessments by MTT Assay

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 been developed for treatment of infectious diseases. Whereas 25 to 50% of existing pharmaceuticals are derived from plants, none are used as antimicrobials. Phytochemicals are one such source from which medicinal plants showing potential therapeutic uses such as anti-inflammatory, antimicrobial and antioxidative activities have a prospective to fill this need because their structures are different from those of the more studied plants. These therapeutically active components obtained from the plant sources, pose different structures although show similar activity. Thus it is very important to study the structure and isolate the phytochemically active compound and check its stability in various factors. The study showed *Ipomea staphylina* posed potential therapeutic activities, however further studies on isolation and characterization of the molecules is required.

Conflicts None declared.

REFERENCES

ALNAJAR, Z.A., ABDULLA, M.A., ALI, H.M., ALSHAWSH, M.A., HADI, A.H. 2012. Acute toxicity evaluation, antibacterial, antioxidant and immunomodulatory effects of Melastoma malabathricum. *Molecules*, 17, 3547-59.

BROUWERS, C., KUPPER, N., PELLE, A.J., SZABÓ, B.M., WESTERHUIS, B.L., DENOLLET, J. Depressive symptoms in outpatients with heart failure: Importance of inflammatory biomarkers, disease severity and personality. *Psychol. Health.*, 2013.

BOMAN, H.G., KALETTA, U. 1957. Chromatography of rattlesnake venom; a separation of three phosphodiesterases. *Biochim. Biophys. Acta*, 24, 619-31.

CHIMIRRI, S., AIELLO, R., MAZZITELLO, C., MUMOLI, L., PALLERIA, C., ALTOMONTE, M., CITRARO, R., DE SARRO, G. 2013. Vertigo/dizziness as a Drugs' adverse reaction. *J. Pharmacol. Pharmacother.*, 4, 104-109.

DALAMAGA, M. 2014. Resistin as a biomarker linking obesity and inflammation to cancer: potential clinical perspectives. *Biomark. Med.*, 8, 107-118.

DUTTA, S., BHATTACHARYYA, D. 2013. Enzymatic, antimicrobial and toxicity studies of the aqueous extract of Ananas comosus (pineapple) crown leaf. *J. Ethnopharmacol.*, 150, 451-457.

FARIAS, D.F., SOUZA, T.M., VIANA, M.P., SOARES, B.M., CUNHA, A.P., VASCONCELOS, I.M., RICARDO, N.M., FERREIRA, P.M.,

RYU, M., MATSUMURA, R., QUAN, G., FURUTA, T. 2013. Comparison of the cytotoxicity of high-level disinfectants by the MTT assay and direct contact assay. *Biocontrol. Sci.*, 18, 221-225.

GALLET, M., VAYSSADE, M., MORRA, M., VERHOEF, R., PERRONE, S., CASCARDO, G. 2009. Inhibition of LPS-induced proinflammatory responses of J774.2 macrophages by immobilized enzymatically tailored pectins. *Acta Biomater.*, 5, 2618-2622.

GUERRA, P., KIM, M., SHAH, A., ALAEE, M., SMYTH, S.A. 2013. Occurrence and fate of antibiotic, analgesic/anti-inflammatory, and antifungal compounds in five wastewater treatment processes. *Sci. Total. Environ.*, 473-474C, 235-243.

HALARIS, A. 2013. Inflammation, heart disease, and depression. *Curr. Psychiatry Rep.*, 15, 400.

HAMSA, T.P., KUTTAN, G. 2011. Evaluation of the anti-inflammatory and anti-tumor effect of Ipomoea obscura (L) and its mode of action through the inhibition of proinflammatory cytokines, nitric oxide and COX-2. *Inflammation*, 34, 171-183.

KATZ, J.A. 2013. COX-2 Inhibition: What We Learned-A Controversial Update on Safety Data. *Pain Medi*, Suppl 1, 29-34.

KIM, J.A., PARK, H.S., PARK, K.I., HONG, G.E., NAGAPPAN, A., ZHANG, J., HAN, D.Y., SHIN, S.C., WON, C.G., KIM, E.H., KIM, G.S. 2013. Proteome analysis of the anti-inflammatory response of flavonoids isolated from Korean Citrus aurantium L. in lipopolysaccharide-induced L6 rat skeletal muscle cells. *Am. J. Chin. Med.*, 41, 901-12.

KIM, S.H., HONG, J.H., LEE, Y.C. 2013. Oleanolic acid suppresses ovalbumin-induced airway inflammation and Th2-mediated allergic asthma by modulating the transcription factors T-bet, GATA-3, RORγt and Foxp3 in asthmatic mice. *Int Immunopharmacol*, 18(2), 311-324.

LAI, R.P., NAKIWALA, J.K., MEINTJES, G., WILKINSON, R.J. 2013. The immunopathogenesis of the HIV tuberculosis immune reconstitution inflammatory syndrome. *Eur. J. Immunol.*, 43, 1995-2002.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L., RANDALL, R.J. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem*, 193, 265-75.

MANVAR, M.N., DESAI, T.R. 2013. Phytochemical and pharmacological profile of Ipomoea aquatica. *Indian J. Med. Sci.*, 67, 49-60.

MELO, V.M., CARVALHO, A.F. 2013. Antibacterial, antioxidant, and anticholinesterase activities of plant seed extracts from brazilian semiarid region. *Biomed. Res. Int.*, Epub 2013, Dec. 10.

MIGNET, N., SEGUIN, J., CHABOT, G.G. 2013. Bioavailability of polyphenol liposomes: a challenge ahead. *Pharmaceutics*, 5, 457-471.

NOLAN, E., O'MEARA, Y.M., GODSON, C. 2013. Lipid mediators of inflammation in obesity-related glomerulopathy. *Nephrol. Dial. Transplant.*, Suppl 4, 22-29.

ORTUÑO, SAHAGÚN, D., MÁRQUEZ-AGUIRRE, A.L., QUINTERO-FABIÁN, S., LÓPEZ-ROA, R.I., ROJAS-MAYORQUÍN, A.E. 2012. Modulation of PPAR-γ by Nutraceutics as Complementary Treatment for Obesity-Related Disorders and Inflammatory Diseases. *PPAR Res*, 2012, 318613.

PRIYADARSHINI, K., NAIDU, K.A., RAGHAVENDRA, R.H. 2013. Screening of Anti-Inflammatory and Anti-Platelet Aggregation Property Studies from Ipomea Staphylina. *Open Access Sci. Rep.*, 2, 714.

REN, K., DUSAD, A., YUAN, F., YUAN, H., PURDUE, P.E., FEHRINGER, E.V., GARVIN, K.L., GOLDRING, S.R., WANG, D. 2013. Macromolecular prodrug of dexamethasone prevents particle-induced peri-implant osteolysis with reduced systemic side effects. *J. Control Release*, 175C, 1-9.

RIDKER, P.M. 2013. Closing the loop on inflammation and atherothrombosis: why perform the cirt and cantos trials? *Trans. Am. Clin. Climatol. Assoc.*, 124, 174-90.

TEKWU E.M., PIEME A.C., BENG V.P. 2012. Investigations of antimicrobial activity of some Cameroonian medicinal plant extracts against bacteria and yeast with gastrointestinal relevance. *J. Ethnopharmacol.*, 142, 265-73.