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Original Research Article

Assessment of antioxidant, antimicrobial and anti-osteosarcoma potential of four traditionally used Indian medicinal plants

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ABSTRACT

In the present investigation *Hibiscus rosa-sinensis* (petals), *Acorus calamus* (rhizome), *Moringa oleifera* (leaves) and *Cucurbita maxima* (petals) were screened for their efficacy against osteosarcoma with elucidating a mechanism of their anticancer potentiality. The methanolic plant extracts revealed the presence of all major phytochemicals with quantitative analysis of flavonoids (98.15 ± 2.02 to 12.34 ± 0.57 mg of RUE/g) and total phenolics (26.40 ± 0.11 to 8.54 ± 0.10 mg of GAE/g). The antioxidant activity was assessed by standard DPPH, H_2O_2 scavenging, NO scavenging assays. The hemolysis, hemagglutination, and erythrocyte aggregation assays unveiled their compatibility with blood components. As most of the opportunistic microbes infect subsequently immunocompromised patients, the antimicrobial activity of the plant extracts showed a zone of inhibition (in mm) against nosocomial strains of *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio cholerae* having MIC between 12.5–50 μg/ml. Through MTT assay the IC_{50} value was calculated against MG 63 osteosarcoma with detailed studies on DNA fragmentation and chromatin condensation, revealing apoptosis being their primary mode of anticancer effect. Further the migration and colony forming assays supported the anticancer potentials of the methanolic plant extracts. The cell cycle analysis revealed that *A. calamus* and *M. oleifera* extracts were capable of arresting the growth of MG 63 osteosarcoma cells.
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Introduction

Plants and civilization go side by side; they are the foundation of various Ayurvedic and therapeutic medicinal formulations. The medicinal property of plants generally depends upon their phytochemical constituents which can be simply described as the chemicals produced by the plants as their secondary metabolites having numerous nutritional and medicinal properties. Over 50% of all modern clinical drugs are of natural product origin and they play an important role in drug development programs in the pharmaceutical industry (Corrêa et al., 2016). Reactive oxygen species (ROS) are the main culprits for the emergence of various health related problems such as cancer, diabetes, cardiovascular diseases and premature aging (Bishayee and Sethi, 2016). These free radicals have the capability of oxidizing nucleic acids, proteins, lipids or DNA and initiate degenerative diseases. Phytochemicals like phenolic acids, polyphenols and flavonoids can scavenge free radicals such as peroxide, nitryl or lipid peroxyl thus inhibiting the oxidative mechanisms that can lead to various degenerative disease conditions (Prakash et al., 2007). Many antioxidant defence systems consisting of enzymatic (superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic (ascorbic acid, glutathione, and α-tocopherol) are present in our body. These compounds have the ability to maintain homeostatic balance during ROS generation, but under conditions of severe stress conditions they alone cannot provide complete protection (Cesaratto et al., 2004). To overcome these sudden outbursts of oxidative stress, natural products from plant sources can provide a feasible substitute to commercially available marketed drugs. Apart from providing relief from oxidative stress these phytochemicals also possess great antimicrobial and anticancer properties which are very important in the present scenario of multiple drug resistant microbes and expensive anticancer therapy (Bonofiglio et al., 2016; Sinha et al., 2016).

The Indian subcontinent is a reservoir of many economically important medicinal plants which forms an integral part of the Indian diaspora. Among a wide range of medicinal plants *Hibiscus rosa-sinensis, Cucurbita maxima, Moringa oleifera* and *Acorus calamus* can be found in almost all households in India. *H. rosa-*

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sinensis commonly known as China rose belongs to the family Malvaceae. Their petals are known to possess hair growth properties; prevent hair loss, premature greying and scalp disorders (Jadhav et al., 2009; Sachdewa and Khemani, 2003). *C. maxima* commonly known as pumpkin belongs to the family Cucurbitaceae, traditionally used as antitumour, anti-inflammatory, anti-diabetic and immune-modulatory agent (Caili et al., 2006). *M. oleifera* commonly known as drumstick belongs to the family Moringaceae. It is predominantly used to curb malnutrition among infants and lactating mothers, in tropics the leaves of Moringa are an excellent source of Vitamin A, B, and C, containing all the essential amino acids, omega 3 & 6 fatty acids and possess anti-diabetic, anti-inflammatory and immunomodulatory activities (Dhakar et al., 2011; Thilza et al., 2010). The sweet flag *A. calamus* commonly known as bacha belongs to the family Acoraceae. Its rhizome is known to possess many medicinal properties against cough, fever, asthma, bronchitis, digestive problems, nerve tonic and expectorant (Liu et al., 2013; Mukherjee et al., 2007).

In the Indian subcontinent the incidence of osteosarcoma is increasing at an alarming rate. Among the various types of cancer, osteosarcoma is the 3rd most commonly occurring cancer which has a bimodal rate of expression having a peak at adolescent period and the next in adult stage (He et al., 2014; Nayak et al., 2016). From literature survey, it was known that phytochemicals in the crude form organize themselves as a group and provide a combined therapeutic effect when compared to individual compounds (Armendariz-Barragan et al., 2016; Saravanan and Aradhya, 2011). Thus keeping into account of the easy availability and traditional therapeutic medicinal values associated with *H. rosa-sinensis* (petals), *C. maxima* (petals), *A. calamus* (rhizome) and *M. oleifera* (leaves) the present investigation was undertaken. There are no previous reports on the efficacy of these plant extracts against osteosarcoma, therefore MG 63 osteosarcoma cells were used to evaluate the anticancer potentials of the above mentioned plant extracts. A detailed comparative analysis regarding their antioxidant potentials and hemocompatibility was designed. As the patients with osteosarcoma undergo a routine schedule of chemotherapy medication procedure, after surgery the patient becomes susceptible to facultative opportunistic microorganisms. Therefore the antibacterial efficacy of the plant extracts against hospital isolate strains of *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio cholerae* was conducted which could be an additional advantage for the patient suffering from osteosarcoma.

Materials and methods

Antibiotic solution (penicillin-streptomycin), Fetal bovine serum (FBS), 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT), Bisbenzimide H-33342 were purchased from Sigma-Aldrich (Mumbai, India), Minimum essential medium (MEM), Dulbecco's Modified Eagle's Medium (DMEM), Folin-Ciocalteu's reagent, Nutrient Agar, Nutrient Broth, 2, 2, diphenyl-1-picryl hydrazyl (DPPH) and all other chemicals of analytical grade were purchased from Hi-media (Mumbai, India). Distilled water was used throughout the experiment.

Collection of the plant materials

Fresh flowers of *H. rosa-sinensis* and *C. maxima,* leaves of *M. oleifera* and rhizome of *A. calamus* were collected from the locality of Rourkela, Odisha, India in the month of August, 2011. The samples were washed gently in tap water followed by distilled water and shade dried. The dried samples were grinded and stored at 4 °C for further use.

Preparation of plant extracts

The plant extracts were prepared following standard protocol (Bhatnagar et al., 2012). Briefly, 5 g of each dried plant samples were mixed with 100 mL of 95% methanol separately in 250 mL conical flask and kept in a rotary shaker for 24 h. The mixture was then filtered using a whatman filter paper and centrifuged at 5000 rpm for 15 min (C24-BL centrifuge, REMI, India), the supernatant obtained were concentrated using a rotary evaporator (IKA® RV 10 rotary evaporator, China). The resultant extracts were further lyophilized (LFD5508, Daihan labtech, India) and kept at 4 °C for further characterization studies.

Qualitative phytochemical analysis

The detection of alkaloids, cardiac glycosides, steroids, terpenoids, saponin, phenols, tannins and flavonoids were carried out following the procedure of Harborne, 1998 (Harborne, 1998). The presences of the specific phytochemical group were designated with (+) sign whereas the absence of the group was indicated with (−) sign.

Quantitative phytochemical analysis

Determination of total phenolic content

Total phenolic compounds in the methanolic extracts of *H. rosa-sinensis, C. maxima, M. oleifera and A. calamus* was determined by Folin-Ciocalteu's calorimetric method (Waterhouse, 2002). Briefly, 0.25 mL of respective plant extracts were diluted with distilled water to make the volume 6 mL and then 1.25 mL of undiluted Folin-Ciocalteu reagent was added to it. After 1 min, 3.75 mL of 20% aqueous Na_2CO_3 was added, and the volume was made up to 25.0 mL with distilled water. The controls contained all the reaction reagents except the plant extract. After 2 h of incubation in room temperature, the absorbance of the resulting blue colour solution was measured by UV–visible spectrophotometer Lambda 35® (PerkinElmer, Waltham, MS, USA) at 760 nm.

Determination of total flavonoids

The flavonoids content in methanolic plant extracts was determined spectrophotometrically (Kumar et al., 2008). Briefly, in a 10 mL test tube 0.3 mL of plant extract was mixed with 3.4 mL of 30% methanol. After vortexing it, 0.15 mL of $NaNO_2$ (0.5 M) was added to the solution and was incubated for 6 min. After incubation 0.15 mL of $AlCl_3{\cdot}6H_2O$ (0.3 M) was added to the above solution and again incubated for 5 min. Subsequently, 1 mL of NaOH (1 M) was added and the final reaction mixture was incubated for 40 min at room temperature followed by taking the absorbance at 415 nm. Total flavonoids content was determined using a standard curve with Rutin (0–100 μg/ml).

ATR-FTIR spectroscopy analysis

The Attenuated Total Reflection Fourier Transform Infrared (ATR- FTIR) spectroscopy analysis was conducted to corroborate the presence of various functional groups associated with the phytochemicals in the methanolic plant extracts. The ATR- FTIR was performed on a Bruker ALPHA spectrophotometer (Ettlinger, Germany) with a resolution of 4 cm^{-1}. The samples were scanned in the spectral region between 4000 and 500 cm^{-1} by taking an average of 25 scans per sample. 1 drop of sample was kept on the sample holder and the samples were scanned and the results obtained were analyzed through OPUS software.

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