A Study of Plant Bioactive Compounds Evaluating Of Anti-Cancer

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Abstract

The aim of this study is to the Plant derived natural products had great promise for the discovery and development of new drugs. Preliminary screening tests are useful in the detection of different secondary metabolites in the plant crude extracts. Extraction with different solvents with increasing polarity influences the phytoconstituents present in the plants. Hence, in the present study different solvents (chloroform, ethyl acetate, methanol, ethanol and aqueous) were used for extraction. The qualitative phytochemical analysis of chloroform, ethyl acetate, methanol, ethanol and aqueous extracts of Ximenia americana, Hopea ponga, Kandelia candel, Vitex leucoxylon and Rhizophora apiculata plants was performed following the standard protocols. In case of X. americana out of five different extracts, presence of alkaloids and lignin were detected in chloroform extract; Phenols, flavonoids and tannins in other 4 solvent extracts except chloroform extract; glycosides were detected in chloroform, ethyl acetate and aqueous extracts; saponins were detected in methanol and aqueous extracts; sterols were detected in chloroform and ethyl acetate extracts; oil and fats were present in all solvent extracts. In case of H. ponga out of five different extracts, presence of alkaloids and lignin were detected in chloroform and ethyl acetate extract; Phenols, flavonoids and tannins were present in methanol extract, ethan<mark>ol</mark> extract and aqueous extract and absent in case of chloroform and ethyl acetate extracts; glycosides and sterols were detected in all the solvent extracts; saponins was detected in chloroform extract. For the K. candel alkaloids were detected in methanol and aqueous extracts; methanol, ethanol and aqueous extracts shows the presence of Phenols, flavonoids and tannins but chloroform and ethyl acetate shows negative result for these phytochemicals; out of five different extracts presence of lignin was detected in ethanol extract; only glycosides were detected in ethanol and aqueous extracts.

Keywords: Plant Bioactive Compounds, Anti-Cancer, natural products, phytoconstituents, chloroform, ethyl acetate extract.

1. INTRODUCTION

Plant resources are used as raw material for different purposes with various applications, they not only provide basic needs of life but also they form a valuable source of phytochemicals. Plant derived natural products have hold great promise for the discovery and development of new drugs. Worldwide more than 30,000 plant species have been used for medicinal purposes. India is a home to thousands of potential medicinal plant species; ranking sixth among 12 mega biodiversity countries of the world. Western Ghats of India represents one of the biodiversity hotspots in the world covering an area (1, 80,000 km2) which is just under 6% of the total land area of India. Western Ghats harbor >30% of all plant, birds and mammalian species found in India. A remarkable number of modern drugs have been isolated from medicinal plants which led to sudden increase in the number of herbal medicines. Medicinal plants of Western Ghats reported with therapeutic properties are used for the treatment of many infectious diseases and severe diseases of humans including cancer as they contain many bioactive Phytoconstituents which are of curative effects. Medicinal plants are backbone of Indian traditional system of medicine. Some of the ethnomedicines have been incorporated in the organized system of medicine, however numerous of ethno medicines have remained untouched especially in western ghat region of Karnataka. Natural products based drugs have been used against various diseases since time immemorial. Plant derived natural products hold great promise for the discovery and development of new drugs. Medicinal plants provided with bioactive compounds which are considered as natural source of antioxidant, antimicrobial and anti-inflammatory agents which have been shown to reduce the risk and progression of many diseases such as cancer and diabetes along with the treatment for parasitic infections in humans and animals. Plants are the major source for discovering new compounds with medicinal value for drug development. Plants are the rich sources of secondary metabolites such as alkaloids, phenols, flavonoids, tannins, saponins, glycosides, terpenoids etc. that possess a wide array of biological properties including antibacterial, antifungal, antioxidant and anticancer. Different parts of plants and whole plant have varied with active compounds and medicinal properties and according to an estimate 80% of the population in developing countries completely depend on traditional medicine for their primary healthcare. However, this demands the screening of medicinal plants for bioactive Compounds as a basis for further pharmacological studies.

2. ANTICANCER

Cancer is a general term applied for series of malignant diseases that may affect different parts of body. This disease is characterized by rapid and uncontrolled formation of abnormal cells, which may mass together to form a tumor or proliferate throughout the body by the process of metastasis. The main types of cancer treatment in humans were surgery, radiation and drugs (chemotherapeutic agents) can often provide temporary relief of symptoms, prolongation of life and occasionally cures. Cancer continues to represent the largest cause of mortality in the world claiming over 6 million lives every year. Cancer is the leading cause of death worldwide and overall statistics study proves that compared to other diseases death rate of cancer is getting very much high every year and it is one of the most common devastating disease affecting millions of people each year. Cancer has been a leading cause of global morbidity due to its rapid progression and poor diagnosis. Lung cancer is the leading cause of cancer deaths in men and 2nd in women. In developing countries since the following decades, numerous people with cancer will continue to increase may be due to life style, nutrition and environmental conditions. In many countries cancer is the 2nd leading cause of death after heart diseases. Lung, colorectal and stomach cancer are among the five most common cancers in the world for both men and women. Lung cancer is the leading cause of cancer deaths worldwide. According to studies conducted by American cancer society estimated in 2016, about 1 of 4 cancer deaths is from lung cancer. Every year, more people die of lung cancer than of colon, breast and prostate cancers. In case of 5 year survival rate of lung cancer patients' metastasis it has dropped to 4% from 54% (Siegel et al., 2016). Smoking is the most important contributory factor in the causation of lung cancer and also certain occupations have shown to increase exposure to lung cancer such as asbestos and textile industries. However, most of the anticancer drugs currently used such as doxorubicin, paciltaxil give rise to undesirable side effects such as cardio toxicity and tumor drug resistance. Many synthetic drugs are available to treat cancer, but those are provided with severe side effects as well as cost effective. According to World Health Organization (WHO) approximately 65-80 % of developing countries including India depend on traditional medicine for their health care due to difficulties of accessing modern medicines. Medicinal plants play a significant role in the treatment of cancer. Natural products or derivatives have been demonstrated to have significant anticancer activities due to their ability to inhibit tumor growth, angiogenesis and metastasis without any side effects. It was reported that, 40 % of anticancer agents between 1940 and 2002 were derived from natural products or their mimics, including vinca alkaloids, Taxus diterpenes, camptotheca alkaloids etc. (David et al., 1999). Since ancient times plant secondary metabolites and their semi synthetic derivatives continue to play an important role in the treatment of cancer as novel drugs and 60% of currently used anticancer agents are derived in one way or another from natural sources. Plant derived natural products such as flavonoids, terpenes, alkaloids and phenols are gaining more importance due to their diverse pharmacological properties including cytotoxic and cancer chemo protective effects. Phytochemicals and even the whole plant extracts are known to prevent arrest or reverse the cellular and molecular processes of carcinogenesis due to its multiple intervention strategies Because of these reasons herbal medicines are making an impact on both world health and international trade. Already large number of new drugs derived from plants secondary metabolites have been applied in treatment and prevention of cancer. In many countries the use of medicinal plants to treat diseases is quiet common due to two main factors i.e. easy access and low cost with less side effects. Surgical resection, radiation or systemic chemotherapy is the main type of treatment for most cancers, but in case of lung cancer post treatment reoccurrence is quite frequent and although the cessation of smoking is important for lung cancer prevention. The preventive mechanisms of tumor promotion by natural Phytochemicals range from the inhibition of genotoxic effects, increased antioxidants and antiinflammatory activity, inhibition of cell proliferation, protection of intracellular communications to modulate apoptosis and signal transduction pathways. The bioactive compounds from medicinal plants are provided with a wide variety of chemical structures with various biological activities and also bioactive compounds from plants are able to suppress or prevent the initial phases of carcinogenesis which provides important prototypes for the development of novel drugs.

In the present study, Ximenia americana, Hopea ponga, Kandelia candel, Vitex leucoxylon and Rhizophora apiculata plants were selected for the study. Leaves of Ximenia americana were collected from Karnatak University

Campus, Dharwad, India in the month of June, 2014, whereas Leaves of Hopea ponga and Vitex leucoxylon were collected from Anashi forest range of Western Ghats, Uttar Kannada District, Karnataka, India during the period of May, 2015 and Leaves of Kandelia candel and Rhizophora apiculata were collected from Mangrove region, Sadashivghad, Karwar, Uttar Kannada District, Karnataka, India during the period of May, 2015.

Phylum/Division: Magnoliophyta Class: Eudicots Order: Santalales Family: Olacaceae Genus: Ximenia Species: X. americana Common Names: Sea Lime, Tallow Wood, Bedara Laut Status: Common	Kingdom:	Plantae	
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	Status:	Common	

Table. 1 Taxonomic classification of Ximenia Americana

Fig. 1. Ximenia americana

3. ANTI-INFLAMMATORY

Inflammation is a very common symptom of many chronic diseases and is normal protective response to tissue injury caused by chemical or microbial agents (Ashley et al., 2012). It is well known fact that, the denaturation of tissue proteins leads to inflammatory and arthritic diseases (Williams et al., 2008). Inflammation is a prominent phenotype of various diseases such as rheumatoid arthritis, atherosclerosis and asthma, although inflammation is primarily a protective response against pathogens, toxins and allergens (Gil, 2002). During an inflammatory response mediator such as cytokines, IL-1, tumour necrosis factor (TNF) and Interferons (INF) are released. There are many synthetic drugs available to treat inflammation but they have disadvantages because of their detrimental

side effects on the gastrointestinal tract, kidneys and on the cardiovascular system and reappearance of symptoms after discontinuation. Steroidal anti-inflammatory agents are available, but they induce damage to the lymphocytes and causes severe side effects. Hence the antiinflammatory agents from natural sources like plants bioactive compounds is gaining importance and they are more promising agent with less side effect. In the present market, the non-steroidal anti-inflammatory drugs are commonly used for the treatment of inflammatory diseases but these are associated with many side effects like gastric irritation, ulcer etc. Since ancient times inflammatory disorders and related diseases have been treated with plant or plant derived formulations, because of their specific action and less side effects. An anti-inflammatory activity of several plant extracts and isolated compounds have already been scientifically demonstrated. Drugs from natural origin are promising and high in demand in management of inflammation conditions due to their fewer side effects and cost effective in nature. In the present study, Ximenia americana, Hopea ponga, Kandelia candel, Vitex leucoxylon and Rhizophora apiculata plants were screened for invitro anti-inflammatory activity by using standard method.

4. ANTIOXIDANT

Reactive Oxygen Species (ROS) are an integral part of normal physiological processes, continuously formed as a consequence of aerobic metabolism in eukaryotic cells. ROS at low-to-moderate concentrations play important role in cell physiology, such as regulation of cell growth, cellular signal transduction pathways, and defense against pathogens. In addition to their biological importance, overproduction of these extremely reactive and unstable oxygen species is considered to be the main contributor to various metabolic and cellular disturbances. Oxidative stress has been suggested to play a major role in the pathogenesis of many degenerative diseases in humans (Halliwell et al., 2007) i.e. inflammatory, cancer, diabetes, aging, cardiovascular diseases; tumor growth and Alzheimer's disease are contributed by increased cell oxidation. Free radicals are the chemical species which contains one or more unpaired electrons. These are highly unstable and attain stability cause damage to other molecules by extracting electrons from them. These free radicals enhance the abnormal uncontrolled oxidation reaction in the body which leads to the failure of antioxidant defense mechanism and causes damage to the cell structures increasing risk factor for many diseases such as Alzheimer's, Parkinson, cardiovascular disorders, liver disease, inflammation and cancer (Rajkapoor et al., 2010). In modern medicine, maintaining the balance between antioxidant defense system and ROS formation is believed to be a critical concept for healthy biological systems (Tiwari, 2001). However, in recently published data there is use of synthetic antioxidants like hydroxyl toluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) in the food industry to prevent oxidative deterioration (Löliger, 1991). But the synthetic antioxidants appear to have carcinogenic and tumor-promoting action (Botterweck et al., 2000). Therefore, it is of great importance to find new sources of safe and inexpensive antioxidants of natural origin in order to use them in food and pharmaceutical formulations. Several studies have showed that increased dietary intake of natural phenolic antioxidants correlate with decreased coronary heart disease (Zadak et al., 2009). Studies have been conducted to identify antioxidants from natural sources, which are suitable alternatives to synthetic antioxidants (Gharibi et al., 2013; Suppakul et al., 2006). Polyphenolic compounds or Phytochemicals present in plants are important components of the human diet; Phytochemicals can be used to regulate oxidation and stress-related chronic diseases such as diabetes and cardiovascular diseases (Kwon et al., 2008). The protective effects of phenolic compounds and flavonoids are directly related to their ability to scavenge free radicals (Fraga, 2010) by undergoing oxidation, producing toxic compounds, which elicit inhibitory effects on pathogenic microorganisms (Vermerris et al., 2006). In order to protect the human body from free radicals, numbers of studies have been carried out on various plants, vegetables and fruits which they are a rich source of antioxidants, such as vitamin A, vitamin C, polyphenolic compounds and flavonoids (Diplock et al., 1998). Natural antioxidants play important role in the enhancement of antioxidant capacity of the blood plasma and helps in the prevention of many diseases including cancer and diabetes (Barros et al., 2007). Phenols and poly phenols are main secondary metabolites present in a plant which acts as antioxidant or free radical scavengers (Nitha et al., 2007). Phenols also known to have several biological activities such as, antiinflammatory, anti-tumor and antioxidant activities (Sulaiman et al., 2011; Schofield et al., 2001) Natural antioxidants derived from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols) ascorbic acid and carotenoids (Ali et al., 2008). Antioxidants are compounds that protect cells against the damaging effect of reactive oxygen species (Deepti et al., 2012). Recently natural antioxidants are in high demand because of their potential in health promotion and disease prevention. It is well known that, the antioxidant properties of the plant extracts cannot be evaluated by single method due to the complex nature of Phytochemicals. In the antioxidant defense system, enzymatic antioxidant such as Superoxide Dismutase (SO), Catalase (CAT), Glutathione Peroxidase (CPx) and non-enzymatic plant derived antioxidants such as

carotenoids, ascorbic acid, phenol, flavonoids etc., are having antioxidant capacity acts as scavengers in the living system. Risk of the chronic disease and its progression can be achieved by increasing natural antioxidant defense or supplementing with the proven antioxidants (Rajkumar et al., 2010). Antioxidant mechanisms in biological tissues are extremely complex and by the single method it is difficult to decide the antioxidant capacity of crude extracts (Carocho & Ferreira, 2013). A large number of in-vitro methods are available to evaluate the antioxidant activity of pure compound or extracts. Hence in the present study, four in-vitro assays viz., FRAP, PM, H2O2 and DPPH assay are used to evaluate antioxidant activity of Ximenia americana, Hopea ponga, Kandelia candel, Vitex leucoxylon and Rhizophora apiculata plants.

5. CONCLUSION

In the present study, total phenolic content of different extracts of Ximenia americana, Hopea ponga, Kandelia candel, Vitex leucoxylon and Rhizophora apiculata leaves was determined by the FCR method and expressed as gallic acid equivalents (GAE) per gram of plant extracts. Aqueous extract of Ximenia americana exhibited highest amount of phenolic content among the extracts i.e. 91.4±0.1414 mg/g of gallic acid equivalent. Variation was observed in total phenolic content in each extracts of plants. Total flavonoids content of different extracts of Ximenia americana, Hopea ponga, Kandelia candel, Vitex leucoxylon and Rhizophora apiculata leaves was determined by aluminum chloride colorimetric method and expressed as quercetin equivalents (QE) per gram of plant extracts. In case of V. leucoxylon out of five different extracts, Alkaloids were detected in chloroform and ethanol extracts; phenols and flavonoids in methanol and aqueous extracts; tannins in ethanol and aqueous extracts; glycosides in chloroform, methanol and ethanol extracts; whereas saponins was absent in all extracts. R. apiculata extracts showed variation in all phytochemicals i.e. out of five extracts alkaloids were detected in chloroform extract; only phenol and flavonoids were detected in methanol and aqueous extracts; tannins were only detected in ethanol extract; glycosides were detected in chloroform, methanol and ethanol extracts; saponins was absent in all solvent extracts.

6. REFERENCES

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