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ORIGINAL ARTICLE

Screening of Phytochemicals and Anticancer Potential of Maclura Pomifera Leaves Extract

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ABSTRACT

Introduction: Maclura pomifera, is small deciduous tree which is commonly called as Osage orange. It typically grows about 8 to 15 meters tall. Maclura pomifera secrete white latex material which is sticky in nature on damaging. The common name of this plant is not related with citrus fruit family "orange" Maclura pomifera belongs to mulberry family called Moraceae.

Method: The extract of Maclura pomifera leaves was prepared by using ethanol as a solvent. The major groups of phytochemicals were present it such as, alkaloids, tannins, saponins, flavonoids and steroids.

Results: The different parts of plants contain, alkaloids, flavonoids, and polyphenolic and many other bio-compound. Due to these compounds, plants posse's antidiabetic, anti-cardiovascular, and anticancer activities. The leaves of Maclura pomifera extract possessed higher cytotoxic potential with IC_{50} 78.9 µg/ml. The cytotoxic potential of extract was checked through MTT assay against the hepatic cancer (HepG2) cells,

Conclusion: These bio-compounds are present in the Maclura pomifera, due to this reason, this plant is used against several disorders such as diabetes, cardiovascular and cancer. Which give the valuable results.

Keywords: Phytochemicals, Anticancer, Cardiovascular, Maclura pomifera, Bio-compounds, Cytotoxic potential

INTRODUCTION

Natural products are those compounds that are manufactured by plants, and many other living organisms. Natural compounds have the ability to induce immunogenic cell death and activate the adaptive immune systems. Natural products have potential against various diseases such as inflammation, diabetes, cardiovascular, and cancer. This potential is due to the presence of bio-molecules in living organisms [1]. Plants are utilized in ancient medication for Millennium of years, have also been continuously evaluated to design new drugs to cure various infectious diseases [2].

Natural plants are fascinating sources to identified new therapeutic, drugs and medicinal agents. Natural plants having anti-tumor, anti-inflammatory, anti-diabetic and anti-cardiovascular efficacy are more affecting because of weaker side effects in comparison to chemically synthesized drugs in laboratories to treat the different disorders [3.] Natural products have bestowed abundant and remarkable innovation in the chasing of recent drug therapies, having numerous therapeutics persuaded by natural products. A significant proportion of present drugs (50%) in the market are natural compounds or derived from them [4,5,6].

Medicines derived from natural sources have been broadly used against various diseases for many years in different parts of the world. These parts of the world are China, Egypt, India, Sumer, and Greece. The worldwide medicine market is almost 1.1 trillion US dollars. And medicines derived from natural sources are about 35 percent [7].

Free radicle are the elements which having free electron, which are actually unpaired, and become the cause of unstable of elements, such as superoxide anion (O_2), hydroxyl radicals (OH), singlet oxygen ($1/2O_2$), and reactive oxygen species have been shown having adverse effects upon cellular functioning in living systems. Free radicals can interact with biological components, resulting in DNA damage, lipid peroxidation, and protein degradation. Excessive generation of free radicals can disrupt the body's prooxidant and antioxidant systems, resulting in a variety of pathological illnesses including rheumatism, arterial hypertension, diabetes, inflammation, neurological diseases, genetic mutations and cancer [8].

Various plant extracts have been declared by researchers as a natural and unlimited source of antioxidants. Excessive formation of free radicals masters natural defensive mechanism, engendering liver injury, as scientists identified an extensive diversity of plant extracts having hepatoprotective effects generally coupled with antioxidant activity. Antioxidants are chemicals that can counteract the adverse effects of free radicals. In biological systems, antioxidants operate as electron donors, free radical scavengers and free catalytic metal chelating agents. Exogenous antioxidants consist of both natural and synthetic substances that have the ability to scavenge free radicals [9].

Maclura pomifera, is small deciduous tree which is commonly called as Osage orange. It typically grows about 8 to 15 meters tall. Maclura pomifera secrete white latex material which is sticky in nature on damaging. The common name of this plant is not related with citrus fruit family "orange" Maclura pomifera belongs to mulberry family called Moraceae. The different parts of plants contain, alkaloids, flavonoids, and polyphenolic and many other bio-compound [10].

The different parts of Maclura pomifera contain, alkaloids, flavonoids, and polyphenolic and many other bio-compound. Maclura pomifera, leaves posse's antidiabetic, anti-cardiovascular, anti-inflammatory, antiviral, antitumor, and anticancer activities. The leaves of Maclura pomifera, are used against several disorders such as diabetes, cardiovascular and cancer [10].

Cancer is the uncontrolled division of cells because no apoptotic processes occur and cells randomly divide. Unidentified mutations occur in cancerous cells and then no apoptosis induced in them and in this way cancerous cells accumulate and then migrate and invade other parts of body [11].

Around 460-370 B.C., a physician named Hippocrates coined the term cancer, which comes from the Greek word "karkinos," which meaning "cancer" [12].

In the 21 centuries, cancer is predicted to be the main reason of death and only critical obstacle for improving life in the world. According to WHO estimates from 2015, the primary or second main cause of death is cancer under the age of 70 years in 91 of 172 countries and ranks 3rd or 4rth in 22 nations. Cancer has remained one of the most difficult diseases to cure since its discovery, and according to data of World Health Organization, cancer is now the 2nd leading cause of worldwide death [13].

Cancer is the uncontrollable division of cells because no apoptotic processes occur and cells randomly divide. Unidentified mutations occur in cancerous cells and then no apoptosis is induced in them in this way cancerous cells accumulate and then migrate and invade other parts of the body [14.] It is a complex heterogeneous disease that is distinguished by a stepwise method. Genetic alterations occur in normal cells that convert into

malignant cells. Then these cells are distinguished by uncontrolled cell growth, immortality, invasiveness, and capacity to form metastasis. Natural products have bioactive compounds that obstruct the cancerous process by changing the behavior of tumor cells and by targeting many signaling pathways [15].

Cancer is a varied variety of tumoral procedures with distinct clinical histories, morphological expressions, grades and pathological phases, as well as diverse sensations to traditional cytotoxic drugs, although having common aberrant modifications [16].

Cancers have troubled multicellular living beings for over 200 million years [17]. Cancer is frequently increasing and leading reason behind death in the whole world [18]. There were calculable 9.6 million cancer deaths and 18.1 million new cancer cases in 2018 all over the world [19].

In 2017, uncommon cancers accounted for roughly 13% of all malignancies diagnosed in individuals aged 20 and above, defined as malignancies with fewer than 6 cases per 100,000 individuals per year [20]. In 2018, cancer and its related pathologies caused almost 10 million deaths worldwide [21].

In 2020 almost 19.3 million new cancer cases diagnosed all over the world, with around 10.0 million cancer deaths. Estimated 2.3 million new cases (11.7 percent), followed by lung (11.4 percent), colorectal (10.0 percent), prostate (7.3 percent), and stomach (5.6 percent) cancers. Lung cancer remained the leading cause of cancer death, accounting for around 1.8 million deaths (18%), followed by colorectal (9.4%), liver (8.3%), stomach (7.7%), and female breast (6.9%) cancers. Due to demographic shifts, the worldwide cancer burden is anticipated to rise to 28.4 million cases in 2040, a 47 percent increase from 2020, with a bigger increase in transitional (64 percent to 95 percent) nations vs transitioned (32 percent to 56 percent) [22].

Ionizing radiation, genetic predisposition, infections, usage of tobacco, poor nutrition, alcohol intake, lazy lifestyle, obesity, and many other environmental exposures are all cancer risk factors. Because of the rise in the prevalence of risk factors, such as obesity and metabolic syndrome, cancer incidence will continue to rise [23]. Cancer is a varied variety of tumoral procedures with distinct clinical histories, morphological expressions, grades, and pathological phases, as well as diverse sensations to traditional cytotoxic drugs, although having common aberrant modifications [24].

Because they are needed for cell growth and survival, cell signaling pathways are the basis of cell communication. Various clinical disorders, such as cancer, are caused by defects in cell signaling or transduction pathways [25]. Oncogenic mutations including impaired regulatory circuits and molecular machinery w regulate cellular function and destiny, endowing tumor cells with a variety of features that aid their malignant activity. The development of tumor cells is characterized by a number of characteristics, including tissue invasion, substantiating angiogenesis, interruption of apoptosis, infinite replication potential, resistance to antigrowth signals, metabolic reprogramming, and genetic imbalance [26].

There are many distinct forms of malignancies, each with its own set of cells that continue to grow, divide, and re-divide rather than dying and producing new abnormal cells. Because of DNA damage, cancer cells originate from normal ones. Cancer is normally shaped as a solid tumor. Some tumors are benign, while others are malignant (non-cancerous). Many forms of cancer can be prevented by making lifestyle changes such as stopping smoking and eating a low-fat diet. Cancer is easier to cure if caught early, and patients have a better chance of living a long life [27].

Aims of the study: Aims of this study were;

- To determine phytochemical analysis of Maclura pomifera leaves extract.
- To investigate cytotoxic potential on Hepatic Cancer (HepG2 cells).

MATERIALS AND METHODS

The present study was done to determine the characterization of phytochemical, antioxidant and anticancer potential of Maclura pomifera leaves extract. In this study following steps were performed:

- Collection of Maclura pomifera leaves preparation of extracts by green technology, phytochemical, MTT assay, and statistical analysis.

Materials: Materials that were used for the research purpose are given below:

Chemicals, and Apparatus: The following chemicals and apparatus were used during the study, the list is given below Glucose, Nutrient agar, Wagner's reagent, Sulphuric acid, Methanol, Ethanol, Ferric chloride, Sodium Hydroxide, Gentamycin, DPPH, (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide), Dimethyl sulfoxide, and Distal water. Microwave oven, rotary evaporator, incubator, weight balance, vortex, petri dishes, test tubes, cotton plugs, cotton swabs, vials, micropipette, aluminum foil, falcon tubes, para film or masking tape, forceps, Eppendorf tubes, beakers, test tubes racks, and discs.

Sample collection and preparation of extract: Maclura pomifera leaves were collected from Quetta, Peshawar, Wah and Lehtrar (near Rawalpindi), Pakistan. Maclura pomifera leaves were grinded through grinder to obtain a fine powder, and then stored in air tight jars. 200 g of powdered material was subjected to flask and 600 ml (80%) ethanol then heated at 400 watts for 6 min then filter it and repeat this cycle for three time. Then rotary that filtrate and crude extracts then lyophilized for 2 to 3 days then these extracts were stored at 4°C for further analysis.

Phytochemical screening: Phytochemical assessment was done to check the presence of bioactive components for example flavonoids, alkaloids, steroids, saponins and tannins in Maclura pomifera leaves [28].

For the screening of flavonoids, we performed the flavonoid test, for this purpose approximately 20 mg of each extract was taken as well as few drops of diluted NaOH were added. After adding few drops of diluted H_2SO_4, yellow color become colorless or disappear approves presence of the flavonoids in extract.

For the screening of saponin, approximately 20 mg of extract was diluted with 20 ml of the distilled water. Then test tube was shaken by hand for 15 min. Foam pattern produced on the top of test tube, which shown presence of saponins in the extract.

For the screening of alkaloids, approximately 20 mg of extract was taken in Eppendorf and dissolved in few drops of 1% picric acid. Appearance of yellowish color precipitates confirms the presence of alkaloid in the extract.

For the screening of steroid, about 20 mg of extract was taken in Eppendorf along with 2 ml of conc. H_2SO_4 added by side walls of test tubes. Dark reddish green color appeared that confirmed the presence of steroids in Maclura pomifera leaves extract.

For the screening of tannins approximately 20 mg of extract was taken in separate Eppendorf and dissolved in 45% ethanol. Then test tube was boiled for 5 min and in test tube 2 ml of 15% ferric chloride solution was added.

Cell culture and Cytotoxic assay: The following steps are performed during the study, firstly Hepatic cancer HepG2 cells were obtained from American Type Culture Collection (VA, USA). These cells were grown in DMEM along with 10% FBS and 20 µl/ml streptomycin. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2.

Firstly, we took 96 well plate and 1×10^6 HePG2 cells were grown in it. Then we incubated these cells overnight for growing before applying different concentrations of Maclura pomifera leaves extract. Taxol was used as the positive control and unpolished media was used as negative control. After 48 h, 20 µg/ml of MTT solution added to every well and incubated for 4h. Triplicates of wells was made to precise our results. After adding 100 µl of DMSO, absorbance was checked through an ELISA

reader at 540 nm. The absorbance of control cells and treated cells was used to examine the extract cytotoxicity. One way ANOVA was applied by SPSS (Statistical Package for the Social Sciences) software on result data to compare mean values and standard deviation was calculated.

RESULTS AND DISCUSSION

A number of activities of the Maclura pomifera leaves extract were checked including phytochemicals analysis for determination of bioactive compounds, and anti-hepatic cancer activity to determine cytotoxic effect of the Maclura pomifera leaves extract.

Phytochemical Analysis: For the determination of presence of various bioactive compounds from the Maclura pomifera leaves extract, the phytochemical screening tests were done. All test clearly confirmed the presence of flavonoids, alkaloids, steroids, tannins and saponins responsible for maintaining different cellular pathways in organisms.

Flavonoids which are also called as bioflavonoids are secondary metabolites with 15 carbon rings in their structure and are excessively found in natural organisms such as, animals, microbes and plants. Flavonoids are the compounds which act as chemical messengers, physiological regulators and cell cycle inhibitors and also involved in many other biochemical cycles. Like various other plants and fruits, Maclura pomifera leaves are also reported to contain flavonoids. To check presence of flavonoids in Maclura pomifera leaves extract was examined. Maclura pomifera leaves extract exhibited positive results for flavonoids by yellow color in test tube which disappeared after addition of few drops of sulphuric acid.

Saponins are the class of compounds abundantly found in animals and plants. These are amphipathic glycosides categorized by the appearance of soap like foam on the surface of the utensils, when shaken in aqueous solution. By structure, these are hydrophilic moieties combined with derivative of steroid or lipophilic triterpene. Maclura pomifera leaves extract diluted by using distilled water in tube and then shaken the tube about 15 min. A clear foam layer formed in test tube of Maclura pomifera leaves extract which confirmed the presence of saponins in it.

Steroids are the biologically active compounds. Steroids have four ringed chains which are specifically arranged. Steroids have two most important functions in the cell: act as signaling molecule and as an important component of cell membrane. Steroid mostly found in animals and some steroids also derived from mushrooms and plants. For detection of steroids, samples were exposed to steroid detection test and the dark reddish color appearance approved the existence of the desired group of phytochemicals in Maclura pomifera leaves extract.

Tannins are the class of biomolecules which form precipitates with proteins and some other compounds such as amino acids. Living organisms are main source of tannins. Tannins are distributed in various species throughout the kingdom Plantae. For tannins indication in Maclura pomifera leaves extract. Maclura pomifera leaves extract was dissolved with 45% methanol and boiled for 5 min then after addition of 1 ml $FeCl_3$ color changed into dark green which confirmed the presence of tannins.

Table 1: Qualitative phytochemical screening of Maclura pomifera leaves extract

Extract	Flavonoids	Saponins	Steroids	Tannins	Alkaloids
Maclura pomifera leaves extract by using ethanol as solvent	+	+	+	+	+

Alkaloids are the naturally occurring compounds usually possessing nitrogen containing hetero cycle. The alkaloids are mostly nonvolatile, colorless and crystalline in nature. It has been found that alkaloids are final product of nitrogen metabolism. Alkaloids are abundantly found in plants such as Maclura pomifera. Maclura pomifera leaves extract displayed yellowish color precipitates in test tube which confirmed presence of alkaloids.

Cytotoxicity assay: In this study, we estimated the cytotoxic effect of Maclura pomifera leaves extract. Maclura pomifera leaves extract against hepatic cancer HepG2 cells. Maclura pomifera leaves extract showed cytotoxic effect against hepatic cancer HepG2 cells. In order to investigate dose-dependent effect of Maclura pomifera leaves extract on the growth of cells, the hepatic cancer HepG2 cells were administrated with different concentrations of Maclura pomifera leaves extract (3.125, 6.25, 12.5, 25, 50, 100, 200 µg/ml) for 48 hours. The results of MTT assay showed percentage viability of HepG2 cells after 48 hours' treatment of extract as 34.618%, 44.678%, 52.726%, 60.557%, 69.545%, 73.198% and 85.369% at 3.125, 6.25, 12.5, 25, 50, 100, 200 µg/ml concentrations respectively Maclura pomifera leaves extract. Inhibitory Concentration IC_{50} of Maclura pomifera leaves extract against HepG2 cell lines was calculated 78.9 µg/ml inhibition in cell growth was measured as compared to untreated control. At 200 µg/ml concentration Maclura pomifera leaves extract percent viability of cells.

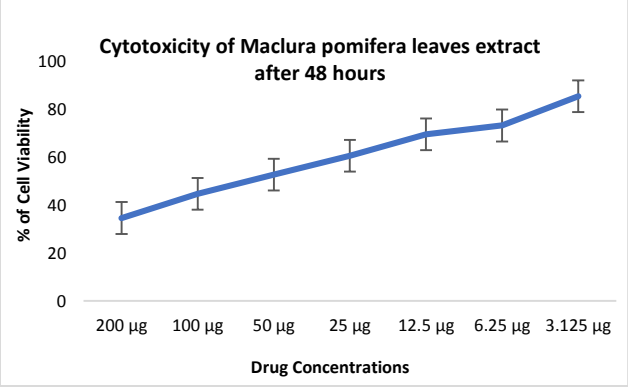

Figure 1: Cytotoxic effect of Maclura pomifera leaves extract against HepG2 cell line after 48 hours treatment

The results of MTT assay showed percentage viability of HepG2 cells after 72 hours' treatment of extract as 28.18%, 37.68%, 42.26%, 47.51%, 58.35%, 66.18% and 75.369% at 3.125, 6.25, 12.5, 25, 50, 100, 200 µg/ml concentrations respectively Maclura pomifera leaves extract. Inhibitory Concentration IC_{50} of Maclura pomifera leaves extract against HepG2 cell lines was calculated 71.7 µg/ml inhibition in cell growth was measured as compared to untreated control. At 200 µg/ml concentration Maclura pomifera leaves extract percent viability of cells.

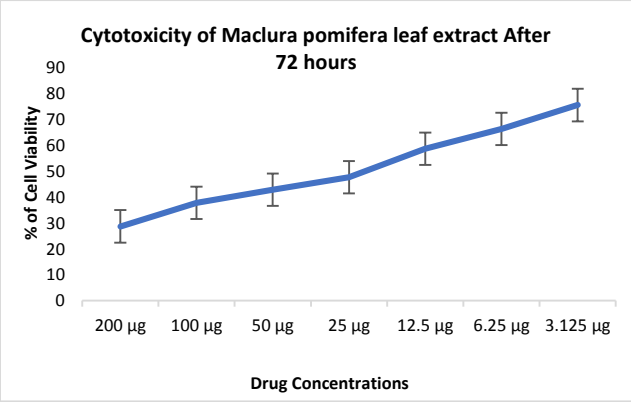

Figure 2: Cytotoxic potential of Maclura pomifera leaves extract against HepG2 cell line at different concentrations after 72 hours treatment.

The results of MTT assay showed percentage viability of HepG2 cells after 48- and 72-hours' treatment of extract at 3.125, 6.25, 12.5, 25, 50, 100, 200 µg/ml concentrations of Maclura pomifera leaves extract. Inhibitory Concentration IC_{50} of Maclura pomifera leaves extract against HepG2 cell lines was calculated 78.9 and 71.7 µg/ml inhibition at 48 and 72 hours respectively in cell growth was measured as compared to untreated control. At 200. Maclura pomifera leaves extract show more results at 72-hours than 48-hours.

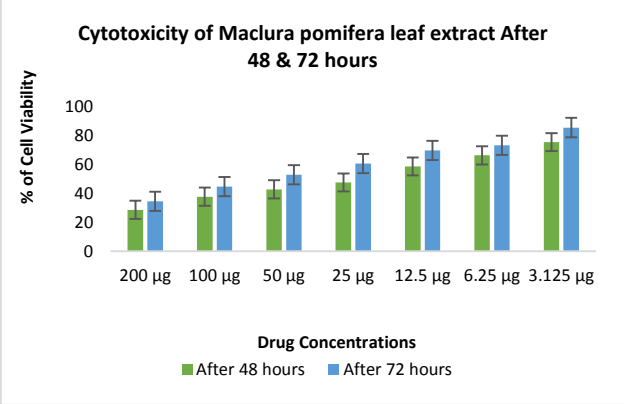

Figure 3: Comparison of cytotoxic potential of Maclura pomifera leaves extract against HepG2 cell line at different concentrations after 48- and 72-hours treatment.

CONCLUSION

Our experimental aims to investigate the phytochemical and anticancer potential of Maclura pomifera leaves extract. The presence of variety of bioactive compounds in this extract has several medicinal and pharmacological properties. The phytochemical analysis of Maclura pomifera leaves extract was checked. The five different compounds belong to specific classes are present in this extract. Maclura pomifera leaves extract displayed anti-hepatic cancer activity through MTT assay.

Future recommendations: The results of our study demonstrate that extracts of Maclura pomifera leaves also should be prepared by using other solvents than ethanol, all bioactive compounds should be identified by using different techniques such as HPLC, TLC and GC-MS. Bioactive compounds of Maclura pomifera leaves extract should be identified and study in vivo model against anti hepatic cancer and other cancer of the different body parts such as lungs, breast, skin and cervical cancer. There is a need to investigate the bioactive compounds of leaves other than Maclura pomifera that are wasted.

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