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PHYTOPHARMACOLOGICAL EVALUATION OF LEAVES OF OLIVE-*OLEA EUROPAEA*

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Abstract

The ethanol extract of *Olea europaea* of the family Oleaceae has been evaluated for the presence of bioactive secondary metabolites by using standard chromogenic reagent and cytotoxicity by brine shrimp lethality bioassay. The ethanol extract of the plant indicated the presence of alkaloids, steroids, flavonoids and tannins. The plant extract exhibited significant cytotoxic effect on brine shrimp lethality bioassay, where the plant extract showed LC_{50} of 263.4 and 13.09 µg/ml after 18 and 24 hours respectively. For the standard carboplatin, LC_{50} were found 70.21 and 0.45 µg/ml respectively after 18 hours and 24hours.

Key words: *Olea europaea,* Secondary metabolites, Cytotoxicity, Brine shrimp lethality bioassay, Carboplatin

Introduction

Olea europaea L. (Family: Oleaceae), Olive in English, locally known as Jolpai, Jolphui, is a medium- to big-sized tree with simple leaves, small flowers in axillary racemes, and one-seeded drupes planted for its edible fruits and timber through out the country[1]. Olive tree (*Olea europaea* L.) leaves have been widely used in traditional remedies in European and Mediterranean countries such as Greece, Spain, Italy, France, Turkey, Israel, Morocco, and Tunisia. They have been used in the human diet as an extract, as herbal tea and a powder and they contain many potentially bioactive compounds that may have antioxidant, antihypertensive, antiatherogenic, anti-inflammatory, hypoglycemic, and hypocholesterolemic properties. One of these potentially bioactive compounds is the secoiridoid oleuropein, which can constitute up to 6-9% of dry matter in the leaves. Other bioactive components found in olive leaves include related secoiridoids, flavonoids, and triterpenes[2]. The leaves of *Olea europaea* are reported to have antioxidant[3], antihypertensive, antiatherosclerotic[4] activity. Some isolated compounds from this tree exhibited antioxidant activities[3-6]. Maslinic acid, a natural triterpene was isolated from *Olea europaea* L. with apoptosis in HT29 human colon-cancer cells via the mitochondrial apoptotic pathway[7]. In this study, the cytotoxic activity of *Olea europaea* was evaluated.

Methods

Collection and identification of plant material

Olea europaea leaves were collected from Narayangonj, Bangladesh in June 2012. The samples of the plant were mounted on paper and the species was taxonomically confirmed by Sarder Nasir Uddin, Principle Scientific Officer, Bangladesh National Herbarium (BNH), Mirpur, Dhaka. A voucher specimen of the plant has been deposited in the library of the same institution and preserved for further collection and reference (DACB-37930).

Preparation of ethanol extract

The collected leaves were separated from undesirable materials. They were dried in open air from 10 to 20 days. The dried plant materials were ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powdered sample was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 265 g of powered plant was taken to a clean, dry, flat- bottomed glass container and soaked in 500 ml of 99% ethanol. Then the container was sealed and kept for a period of 10 days with occasional shaking or stirring[8]. The whole mixture then underwent a coarse filtration by cotton. It was then filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate was concentrated under air. It rendered 8 g of greenish black color and was designated as crude ethanol extract.

Chemicals and reagents

Standard chromogenic reagents used for chemical group test were of reagent grade and purchased from Sigma-Aldrich Co. LLC, Missouri, United States. Carboplatin, used as a standard drug in the cytotoxic assay was collected from the Techno Drugs Limited, Bangladesh. Ethanol supplied by Laboratory Patterson Scientific, U.K. was used as solvent. Dimethyl sulfoxide (DMSO), ≥99.9% purchased Sigma-Aldrich, India was used as solvent to dissolve the extracts. Sodium Chloride Crystal GR from Merck Ltd., Mumbai, India was used to prepare sea water in brine shrimp lethality bioassay.

Brine Shrimp

Brine shrimp eggs were purchased from Carolina Biological Supply Company, Burlington, NC, USA and nauplii were obtained by hatching brine shrimp (*Artemia salina*) eggs.

Instruments and equipment

Electronic balance (serial no.-1508, OHAUS, Germany) was used for this study. Glass made hatching tank, air pump and cover lamp to grow shrimp were purchased locally. Pipettes, Micro-pipette, test tubes and other glass apparatus used were of laboratory standard and procured from authorized dealer.

Test for different chemical groups

The preliminary phytochemical screening of the crude ethanol extract of *O. europaea* was carried out by using standard chromogenic reagents- lead acetate, potassium dichromate, ferric chloride, hydrochloric acid, sulphuric acid, Mayer′s reagent, Dragendorff's reagent, Wagner′s reagent, Hager's reagent, Molisch's reagent, Benedict's reagent and Fehling's solutions were used to detect steroids, alkaloids, gums, flavonoids, saponins, tannins, and reducing sugars using standard protocol[9]. The colour intensity or the precipitate formation was used as analytical responses to these qualitative tests. 10% (w/v) solution of the extract in methanol was used for each of the above test.

Test for cytotoxic activity

The cytotoxicity assay was performed on brine shrimp nauplii using the modified method of Mayer *et al*[10] determining the 50% lethal dose (LC_{50}) and 90% lethal dose (LC_{90}) of the extract. Brine shrimp nauplii were obtained by hatching brine shrimp eggs in artificial sea-water (3.8% NaCl solution) for 22 hrs. Sample was prepared by dissolving of 100 mg of plant extract in 10ml of artificial sea water containing Dimethyl sulfoxide (DMSO) to have concentration of 10 µg/µl. From this solution 1, 5, 10, 50, 100 and 500 µl were transferred to each 10 ml vial and using artificial sea water. Volume was adjusted to 10 ml water to give concentrations of compound of 1, 5, 10, 50, 100 and 500 µg/ml respectively. Brine shrimp nauplii were grown in these solutions and observed their mortality at 18 and 24 hours. The resulting data were transformed to probit analysis software (LdP Line software, USA)[11, 12] for the determination of LC_{50} and LC_{90} values of the extract and standard. Artificial sea-water medium containing DMSO used for the analysis was employed as negative control. Carboplatin was used as standard in this assay.

Results

Chemical group test

Different chemical tests on the ethanol extract of *Olea europaea* showed the presence of steroids, alkaloids, flavonoids and tannins (Table 1).

Cytotoxic activity

Table 2 shows the cytotoxic effect of the ethanol extract *O. europaea* using brine shrimp lethality bioassay. In the test, the extract showed LC_{50} of 263.4 and 13.09 µg/ml after 18 hours and 24 hours respectively. Where, standard carboplatin showed LC_{50} of 70.21 and 0.45 µg/ml after 18 hour and 24 hours respectively (fig. 1). An approximate linear correlation was observed when concentrations versus percentages of mortality were plotted on graph paper.

Discussion

As certain groups of plant secondary metabolites like tannin, reducing sugar, alkaloid, flavonoid, gum, saponin and steroidal compounds are responsible for some specific pharmacological actions, the phytochemical screening of the *O. europaea* were carried out[13].

Cancer, malignant tumour or neoplasm is a broad term for a large group of diseases that can affect any part of the body. 8.2 million people worldwide died from cancer in 2012 and 60% of world's total new annual cases occur in Africa, Asia and Central and South America[14]. From the beginning of history, natural products have afforded a wealthy source of compounds that have found many applications in the fields of medicine, pharmacy and biology. Within the sphere of cancer, a number of significant new commercialized drugs have been obtained from natural sources, by structural alteration of natural compounds, or by the synthesis of new compounds, designed following a natural compound as model. The search for improved cytotoxic agents continues to be a key line in the finding of modern anticancer drugs. The enormous structural diversity of natural compounds and their bioactivity potentials have meant that several products separated from plants, marine flora and microorganisms can serve as "lead" compounds for development of their therapeutic potential by molecular modification. Furthermore, semisynthesis processes of new compounds, obtained by molecular modification of the functional groups of lead compounds, are able to produce structural analogues with superior pharmacological activity and with lesser side effects. Even today, over 60% of the 140 plus agents presently available in Western medicine can trace their provenance to a natural-product source[13].

To evaluate the traditional exercise in tumour, the extract was underwent brine shrimp lethality bioassay using *Artemia salina* comparing with standard anti-cancer drug carboplatin. Among the methods to assay antitumour and cytotoxic activities namely brine shrimp lethality assay, cytotoxicity against cultured cells, Sulphorodamine B (SRB) assay, MTT assay, *Agrobacterium tumefaciens* induced potato disc assay etc.; brine shrimp lethality bioassay is an easy, non-expensive[13, 15] and straight forward bench top screening method for predicting important pharmacological activities like enzyme inhibition, ion channel interference, antimicrobial and cytotoxic activity[10, 16]. In the present study the extract showed LC_{50} at a very low concentration indicating that the extract is significantly potent. Ideally, any agent useful in the treatment of cancer should not be toxic to normal cell. However, in reality, anticancer agents are often toxic to normal cells, particularly towards rapidly growing cells and this plant previously showed anticancer property[7].

There has been a great deal of interest of late in the role of complementary and alternative drugs for the treatment of various acute and chronic diseases like cancer. Among the several classes of phytochemicals, interest has focused on the antioxidant properties of the polyphenols that are found in various botanical agents. Plant vegetables and spices used in folk and

traditional medicine have gained wide acceptance as one of the main sources of prophylactic and chemopreventive drug discoveries and development[13]. Some isolated compounds from this tree exhibited antioxidant activities[3-6]. Maslinic acid, a natural triterpene was isolated from *Olea europaea* L. with apoptosis in HT29 human colon-cancer cells via the mitochondrial apoptotic pathway[7]. Antioxidant polyphenols present in *O. europaea* may contribute to the cytotoxic and anticancer properties of the plant. Advanced studies could be carried out to get a bigger picture to elucidate the correlation of the bioactivities with the mechanism of action of the reported and other possible chemical constituents present in the plant and correlate the. Subsequent development and clinical trials will facilitate to found a new drug candidate for human use.

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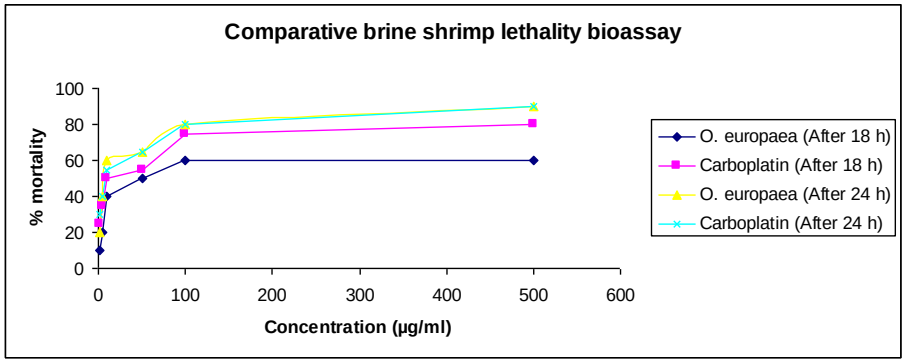

Figure 1. Comparative brine shrimp lethality bioassay of the ethanol extract of *Olea europaea* with standard carboplatin

Phytoconstituents	Ethanol extract of *Olea europaea*
Alkaloids	+
Steroids	+
Carbohydrates	-
Flavonoids	+
Gums	-
Saponins	-
Tannins	+

+: Positive result; - : Negative result

Table 1. Results of phytochemical screening of *Olea europaea* extracts.

Sample	**After 18 hrs**	**After 24 hrs**	**Regression equation**	
	LC_{50} (µg/ml)	LC_{50} (µg/ml)	After 18 hrs	After 24 hrs
O. europaea L. extract	263.4	13.09	y = 0.0656x + 32.724	y = 0.0936x + 48.775
Carboplatin	70.21	0.45	y = 0.0818x + 44.257	y = 0.0905x + 49.959

Table 2. Brine shrimp lethality bioassay of the ethanol extract of *Olea europaea*