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Antitumour activity of essential oil of rosemary by potato disc method

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Abstract

A study was carried out to assess the antitumor activity of essential oil of rosemary (*Rosmarinus officinalis* L.) by potato disc method. Regression analysis revealed highly significant (P≤ 0.01) differences among various concentrations of rosemary oil regarding tumour count and percent tumour inhibition in potato discs. A linear increasing trend was observed for inhibition percentage with an increase in oil concentration while a linearly decreasing trend was depicted for tumour count with increasing concentrations of rosemary oil. The highest inhibition (42.85%) was observed for 35 ppm essential oil which was statistically at par with 30ppm concentration (42.5%). Lowest inhibition was recorded for 10 ppm (15.29%). The instant results suggest that potato disc antitumor assay could be used as a reliable, inexpensive and a rapid pre-screen for antitumor activity of rosemary oil or similar extracts from other plant species.

Key words: rosemary, essential oil, potato discs, tumours, antitumor

1. Introduction

The nutritional, medicinal, anti-microbial and other properties of Rosemary (*Rosmarinus officinalis* L; Family: *Lamiaceae*) have been well known to mankind for thousands of years. Cultivation and use of the species has been reported from ancient Egypt, Mesopotamia, China and India (Bradley, 2006). Native to the Mediterranean region, it is cultivated in temperate climates (Chomchalow, 2002). It is a cool loving, fragrant, leafy and evergreen shrub. The plant can attain a height of upto 2m. It prefers sandy soils and responds well to application of nitrogen and lime. The crop is suitable to cultivate in temperate Himalayas (Mishra *et al.,* 2009). The medicinal importance of rosemary has been well recognized and documented in modern times (Ghannadi, 2002; Al-Sereitia *et al.,* 1999; Bradley, 2006). A variety of therapeutic and medicinal properties have been attributed to the oil, leaves or extracts of this species including being anti-hypertensive, antibacterial, anti-fungal, anti-inflammatory, a nerve tonic, astringent, diaphoretic, stimulant, carminative, spasmolytic, thymoleptic, sedative, diuretic, rubefacient and analgesic. It is traditionally used as a spice in foods, beverages and as alternative herbal medicine for GI ailments including flatulence and dyspepsia. It has been found useful in headaches, myalgia, sciatica, intercoastal neuralgia, renal and biliary colic or liver and gallbladder complaints among other ailments Rosemary oil has also been used in balneotherapy and aromatherapy. (Al-Sereitia *et al.,* 1999; Gilani, 2005; Nusier *et al.,* 2007; Issabeagloo *et al.,* 2012).

In recent years' rosemary oil has not only been used as an ingredient in the preparation of Eau-de-Cologne (Moss *et al.,* 2003) but the food industry has also shown increasing interest in the antibiotic and antioxidant properties of its essential oils and organic or aqueous extracts. The industry is interested in using rosemary extracts as natural preservative instead of synthetic ones to attract the "green" consumers (Davidson, 2005; Peiretti *et al.,* 2012). Rosemary extract is considered one of the most important sources of phenolic compounds with strong antioxidant activity due to their phenolic hydroxyl groups (Raskovic *et al.,* 2014). Rosemary oil is also known to possess anti-carcinogenic activities and chemo-preventive properties (Al-Sereiti *et al.,* 1999; Aherne *et al.,* 2007).

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Despite the many medicinal and therapeutic properties, the specific bioactive ingredients of rosemary responsible for particular therapeutic effects have not been characterized in detail for their chemical nature and mechanism of action though valuable progress has been reported regarding chemistry of the essential oils (Doolaege *et al.,* 2007, Ozcan and Chalchat 2009; Jiang *et al.,* 2011). Keeping in view the potential of anti-carcinogenic potential of rosemary oil, a reliable, less expensive pre-screen method is needed for assessing antitumor activity of the rosemary essential oil and other similar oils. The present study reports the findings of using potato disc method for testing the efficacy of rosemary oil for its antitumor activity.

2. Materials and methods

An experiment was conducted during 2013-14 at the Institute of Agri-Biotechnology & Genetic Resources (IABGR), National Agricultural Research Centre (NARC), Islamabad, Pakistan to assess the anti-tumour activity of essential oil of rosemary through potato disc method described by Ahmad *et al.,* (2008). The experimental materials were obtained from Seed Health Laboratory of NARC.

2.1. Extraction of essential oil

The essential oils were extracted from shade dried and powdered rosemary leaves by hydro-distillation method using Clevenger apparatus. Different concentrations (10, 15, 20, 25, 30 and 35 ppm) of essential oils were prepared in Dimethyl sulfoxide (DMSO). All the solutions were prepared and inoculation or culturing was done using sterile techniques in an aseptic environment under laminar flow.

2.2. Assay for anti-tumour activity

The anti-tumour activity of rosemary oil was assayed at six different concentrations (10, 15, 20, 25, 30 and 35 ppm) for tumour inhibition in the potato discs normally produced by *Agrobacterium tumifaceins* (LBA- 4404), a gram negative and tumour inducing, soil bacterium.

2.3. Preparation of media, culture and inoculum

A. tumefaciens was cultured in Luria broth (LB) media prepared by dissolving 2 g powder in 100 ml of distilled water and adjusting the pH to 7.0 followed by autoclaving (121ºC and 15 psi pressure). After cooling the media was inoculated with a loop full of single colony culture of *A. tumefaciens* (LBA-4404) and incubated for 48 hours at 37Cº in shaking incubator. Inoculum (1500 µL) was prepared by adding 150 µL of essential oil, 750 µL of autoclaved distilled water and 600 µL of bacterial culture in sterile Eppendorf tubes. Positive control was prepared by taking 750 µl of autoclaved distilled water and 750 µl of bacterial culture in sterile Eppendorf tubes. Six plates each, containing 2% Agar as supporting media for the potato discs, were prepared for each concentration of essential oil and each control. These plates were prepared by pouring a 100 ml of 2% autoclaved agar in each autoclaved 9cm petri-plate and allowing solidifying.

2.4. Assay procedure

Red skinned potatoes were thoroughly washed under running tap water and then surface sterilized in 0.1% mercuric chloride ($HgCl_2$) solution in distilled water for 7-10 minutes, followed by rinsing with autoclaved distilled water. Cylindrical potato slices were obtained with the help of sterilized cork borer (8 mm). From both ends of the cylinder, 1cm slices were cut off with sterilized blade and discarded. The remaining cylinder was cut in to 5 mm × 8 mm thick discs which were placed on solidified agar plates (10 discs per plate). One drop of inoculum (50 µl) including appropriate concentration of the essential oil was applied to the surface of each disc and allowed to diffuse for 10-20 minutes into the discs. The plates were then covered and wrapped in parafilm strips to avoid contamination and loss of moisture during incubation. The plates were incubated at 28ºC for 21 days.

2.5. Data collection and statistical analysis

After incubation, the potato discs were placed on slides and examined under the microscope Nikon UFX-DX to count the tumours (Fig. 1). Lugol's solution, prepared in distilled water (10% KI, 5% I_2), was used for staining the discs for 30 minutes. The Lugol's reagent reacted with the starch in the potato discs and turned them into dark brown to dark blue colour. However, the tumours induced by *A. tumefaciens* appeared orange to creamy because they do not take up the stain (McLaughlin *et al*., 1998). Numbers of tumours per disc were counted and percentage inhibition for each concentration was determined as follows;

$$\text{Percentage inhibition} = \left[\frac{\text{Average no of tumours of sample}}{\text{Average no of tumours of} - \text{ve control}} \times 100 \right]$$

The data on tumour count in test and control samples and percent tumour inhibition was subjected to regression analysis as suggested by Gomez and Gomez (1984).

3. Results

3.1. Percent inhibition of tumours

The mean values of percent inhibition of tumour at various concentrations of rosemary oil are presented in figure 2 (a). Regression analysis revealed significant ($P \leq 0.05$) increasing trend in percent tumour inhibition with an increase in concentration of rosemary oil. The increasing trend was linear, thus maximum percent inhibition was recorded with the treatment using 35 ppm rosemary oil. Minimum inhibition was recorded in potato discs treated with 10 ppm rosemary oil. This increased inhibition of tumour might be attributed to the enhanced antitumor activity of rosemary oil due to increase in its concentration. Anti-carcinogenic and chemo-preventive properties of rosemary oil have previously been reported by Al-Sereiti *et al.,* (1999) and Aherne *et al.,* (2007). In the current experiment too, the crude extract of essential oil exhibited a pronounced level of tumour inhibition, which might be due to the antagonistic effects of the compounds present in the crude extract. However, Inayatullah *et al.,* (2007) had found that the effect of crude extract of *H. nepalensis* on viability of *A. tumefaciens* was quite insignificant indicating that the extract/fractions are not involved in killing the bacterium (*A. tumefaciens*) that causes tumours, but rather inhibit tumours by other means. The same mechanism may be at work in case of rosemary oil too. This may become clear with the on-going experiments in our lab.

3.2. Average tumour count

The average number of tumours as recorded in various concentrations of rosemary oil is presented in figure 2 (b). Regression analysis revealed highly significant ($P \leq 0.01$) decrease in average tumour with an increased concentration of rosemary oil. The decreasing trend was linear and thus maximum tumours (7.2) were recorded at 10 ppm whereas minimum was recorded at 35 ppm (figure 1, b). This decrease in tumour count might be due to the antitumor performance of rosemary oil. These findings are in complete agreement with the previous results of Inayatullah *et al.,* (2007). whose findings support the present results. Similarly the tumour counts were recorded in tested as well as in positive control samples. Figure 2 (c) clearly depicts that in control samples the tumour count was statistically same and no significant differences for tumour were observed. In contrast the tumour counts significantly decreased in tested samples with increased concentration of oil. The instant results for the potato disc antitumor assay supports the previous findings that rosemary plant has highly potent antitumor agents.

4. Conclusions and discussion

The instant results for the potato disc antitumor assay suggests that rosemary plant has highly potent anti-tumour agents and thus highest inhibition was observed for 35 ppm essential oil (42.85%), whereas lowest inhibition was recorded for 10ppm (15.29%). Present results suggest that potato disc antitumor assay could be used as a reliable, inexpensive and a rapid pre-screen for antitumor activity. As reported by Mishra *et al.,* (2009), the rosemary plants can be successfully cultivated in temperate Himalayas. It means that rosemary can be successfully cultivated in certain northern parts of Pakistan which may bring additional income to the farmers.

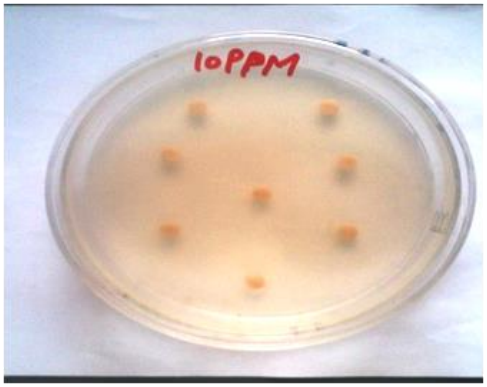

Figure 1. Potato discs placed on 2% agar to determine anti tumour activity of different concentrations of rosemary essential oil

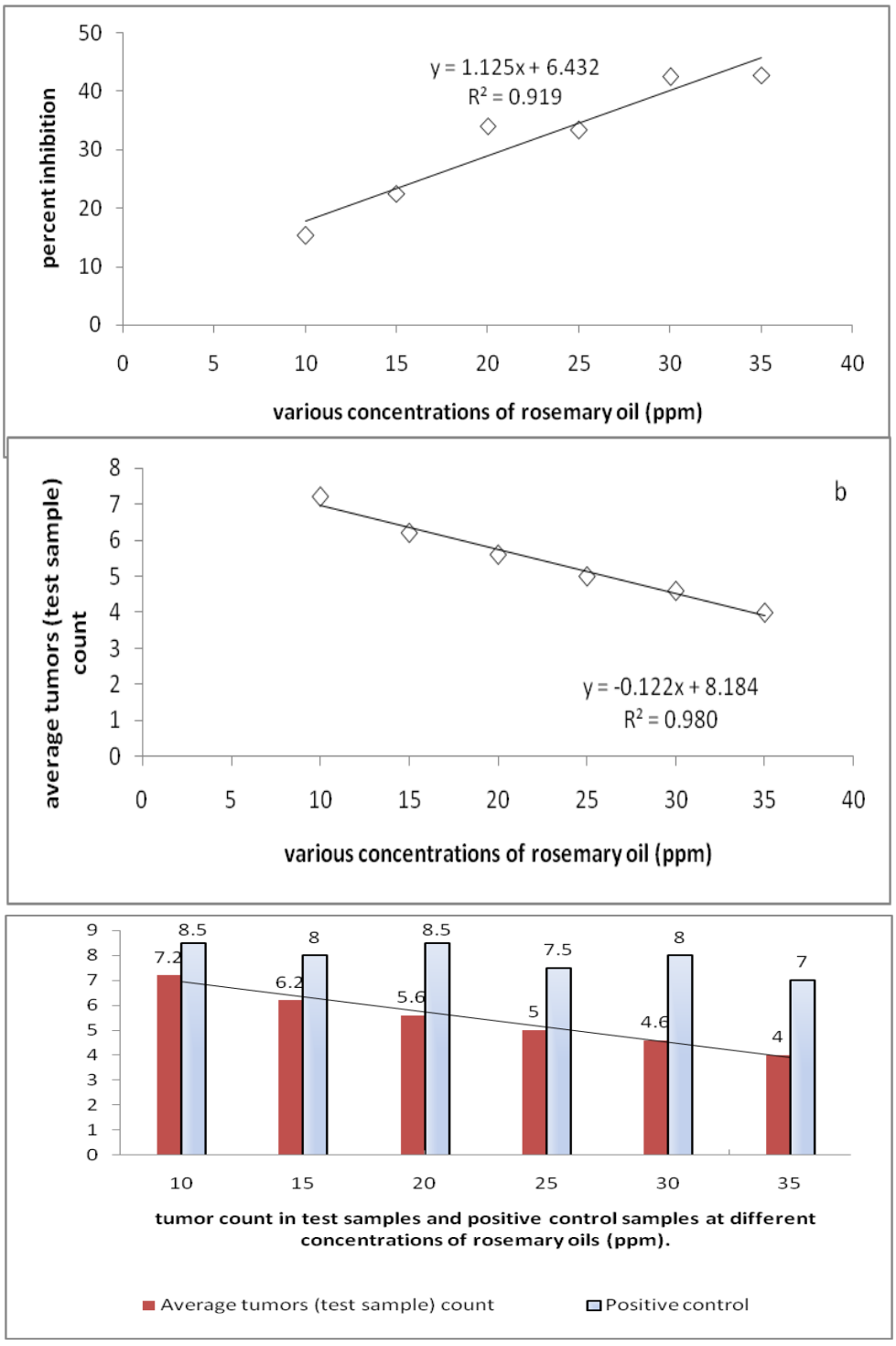

Figure 2. (a) Percent tumour inhibition on potato discs using different concentrations of rosemary oil (b) Average tumour count in test samples plotted against various concentration (c) Tumour count in test and control samples at various concentrations of rosemary oil.

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