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SCREENING OF MEDICINAL AND AROMATIC PLANTS OF HIMACHAL PRADESH FOR ANTIPROLIFERATIVE ACTIVITIES AGAINST BUDDING YEAST *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Medicinal plants contain a lot of bioactive compounds having various activities against human diseases. Based on traditional knowledge 62 plants (including 46 medicinal, 2 aromatic and 14 non medicinal plants) belonging to 38 families were collected from different regions of Himachal Pradesh. The plants from Himalayan region were tested for their anti-proliferative activities by using *Saccharomyces cerevisiae* as a drug discovery tool. The ethanolic extracts and essential oils of different plant parts such as flowers, leaves, fruit and rhizome were tested for anti-proliferative activity against budding yeast using agar well diffusion assays. Amongst the tested plants, *Curcuma longa* (rhizome) (Zingiberaceae) showed highest anti-proliferative effect with a zone of clearance of 26 mm and found fungicidal for yeast growth. *Chrysanthemum indicum* (leaves) (Asteraceae) exhibited anti-proliferative activity, with zone of clearance of 19.5 mm and fungistatic effect. In comparison, *Aegle marmelose* (leaves) (Rutaceae) showed least anti-proliferative effect, with zone of clearance of 18.5 mm and found fungicidal. The essential oils of *Cymbopogon citratus* and *Rosmariuns officinalis* also exhibited strong anti-proliferative effects against yeast growth. The phytochemicals of these plants responsible for anti-proliferative effect in yeast can be purified and explored for anticancer potential in humans.

KEYWORDS: **yeast, anti-proliferative, anti-cancer, cell cycle, medicinal plants, phytochemicals, Himachal Pradesh, *Saccharomyces cerevisiae***

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INTRODUCTION

Plants serve as a valuable resource for the isolation of novel bioactive compounds to combat various human diseases[1]. There are several plants which have been used since ancient times as natural product for treating diseases by village folk[2]. The phytoconstituents isolated from plants have chemical substances which bind to effective target sites in human body[3]. The most important bioactive compounds from plants are alkaloids, glycosides, terpenoids, steroids, flavanoids[4], tannins, saponins, sugars, cumarins[5], proteins, and phenols[6]. Medicinal plants have been used for treating communicable as well as non communicable diseases. Human communicable diseases caused by bacteria, fungi and viruses are the leading cause of deaths in developing countries[7]. Several phyto-compounds and essential oils have been reported from medicinal and aromatic plants for their anti-microbial action against bacterial and fungal pathogens[8]. However, they have not been explored much for treating non communicable diseases like cancer. Of the 250,000 -500,000 species found on earth, ~ 3000 plant species have reportedly been used in treatment of cancer[9]. Since 1990, the National Cancer Institute (NCI), USA has screened more than 60,000 compounds against different human cancer cell lines[10]. Almost 75% of the anti-cancer drugs prescribed are derived from medicinal plants[11]. The most common and extensively used plant derived anti-cancer drugs include vinblastine, vincristine, taxanes, and campothecins[12]. Most of the plant derived bioactive compounds used in anticancer therapy are discovered through large scale screening programs, which are time-consuming and less economical[13]. The present study was conducted to explore the medicinal and aromatic plants of Himachal Pradesh (H.P.) for anti-proliferative activity by using budding yeast *Saccharomyces cerevisiae* as a drug discovery tool. The hill state of India, Himachal Pradesh is very rich in plant diversity, including medicinal and aromatic plants. Medicinal plants are used by village folk to cure many diseases[14]. However, there are no reports regarding exploration of medicinal plants of H.P. for anti-proliferative activities. Budding yeast is being used as a screening tool for the identification of various bioactive molecules in a variety of biological processes[15]. Its genome is well sequenced and the existing knowledge of the fundamental processes in yeast is useful for drug discovery. Many cellular processes, including the cell cycle machinery are highly conserved between yeast and humans[16].The budding yeast offers an excellent biological system for such large-scale screening owing to its amenability for rapid growth screen and rich repository of knowledge about cell cycle regulation. Thus, the anti-proliferative compounds identified from yeast are directly relevant for anti-cancer drug discovery in humans, owing to the conservation of cell cycle machinery between yeast and humans, the regulation of which is perturbed in cancer.

MATERIALS AND METHODS

Yeast strain and growth conditions

Wild-type *S. cerevisiae* strain of W303 background (*MATa ura3 can1*) was used for the study. The yeast strain was cultured in YPD media and grown at 30^{o}C.

Collection of plant material

The medicinal plants and their parts (leaves, root, bark, rhizome and flowers) were collected from the following districts of Himachal Pradesh during the season of availability: Solan (altitude 1350 m, temperature 20-30^{o}C), Hamirpur (altitude 738 m, temperature 30-35^{o}C), Una (altitude 369 m, temperature 33-38^{o}C), Mandi (altitude 1044m, temperature 23-29^{o}C). These plants were identified from the herbarium of Dr Y.S. Parmar University of horticulture and Forestry, Nauni, Himachal Pradesh.

Preparation of plant extracts

The plant material used for the study was washed with tap water to remove dirt and soil particles, surface sterilized with 1% H_2O_2 for 10 minutes, and rinsed with sterile distilled water. The sterilized plant material was air dried (1 week) or dried in oven at 40^{0}C for 4-5 days.

Dried plant material was crushed in mortar and pestle to form a fine powder. The fine powder (4 g) was extracted in soxhlet apparatus using ethanol as solvent[17]. The extract was dried under vacuum in a rota-evaporator at 40°C and stored at -20°C until further use. The extracts were resuspended in ethanol to a concentration of 0.4 mg/µl for performing anti-proliferative assays.

Anti-proliferative assay for S. cerevisiae

The anti-proliferative effect of plant extracts on *S. cerevisiae* growth was studied by agar well diffusion assay[18 19]. An overnight grown culture of yeast cells (~3× 10^8 cells) was mixed with molten YPD soft agar (1% agar) and overlaid on precast YPD hard agar (2% agar). The wells were punched with a cork borer (10 mm diameter) and plant extract (24 mg) was loaded into the wells. The assay plates were incubated for 24 h at 30°C. The zone of clearance (mm) was calculated as follows: diameter of zone of clearance of yeast growth measured in two directions and the average of the two values was taken. The standard error was calculated from the results of two independent experiments. To assay the fungicidal or fungistatic nature of anti-proliferative effect of the plant extracts, the cells were carefully scraped from the zone of clearance of yeast growth around the wells and streaked on YPD agar plates and assayed for growth after 48 h of incubation at 30°C.

Phytochemical analysis of plant extracts

The plant extracts exhibiting anti-proliferative effect on yeast growth were tested for the presence of alkaloid, glycosides, terpenoids, steroids, flavanoids, tannins, saponins, reducing sugar, coumarins, proteins and phenols. Phytochemical tests were performed as described[20 21].

RESULTS

The present study was conducted for determining the antiproliferative activity of the medicinal and aromatic plants of Himachal Pradesh, using the budding yeast *S. cerevisiae* as a screening system. The selection of traditional medicinal plants for this study was based on their reported use in cancer treatment with scientific literature or folklore medicine used by the local population of Himachal Pradesh. A total number of 62 plants (including 46 medicinal, 2 aromatic and 14 non-medicinal plants) belonging to 38 different families were collected from different regions of Himachal Pradesh. Different plant material such as leaves, fruit, flower, bark, bulb, rhizome, seeds and stem were collected and their ethanolic extracts were tested for their effect on the growth of *S. cerevisiae* by using well-diffusion assay. Interestingly, several of these plant extracts inhibited the growth of yeast cells, thereby producing zone of clearance around the respective well, which was measured for determining the extent of anti-proliferative activity (Fig.1A). The effect was specific to the tested plant extracts, since solvent alone (control) failed to produce any zone of clearance (Fig.1A). A representative result of anti-proliferative assays are shown in Fig 1A. Amongst the plants tested, extracts from 13 different families of plants were found to exhibit anti-proliferative activity (Table 1). The ethanolic extracts of 17 plants and essential oil of 2 aromatic plants exhibited anti-proliferative properties on the growth of *S. cerevisiae*, while the extracts of remaining 43 plants failed to exhibit any detectable anti-yeast activity (Table 1). On the basis of the anti-proliferative activities quantified by the magnitude of the zone of clearance of yeast growth, the plants were categorized into three classes; 1) Class I- The plants whose extracts showed zone of clearance in the range of 18-26 mm; 2) Class II- Plants extract showing zone of clearance below 18 mm; 3) Class III- Plants whose extracts had no detectable antiyeast activity. The class I plants with strong anti-proliferative activity include *Curcuma longa* (rhizome), *Chrysanthemum indicum* (leaves), *Aegle marmelose* (leaves, fruit) and *Cymbopogon citratus* (oil), with zone of clearance of 26±0.2, 19.5±0.2, 18.5±0.2 and 19±0.2 mm respectively (Table 1). The class 2 included 15 plants whose extracts showed zone of clearance in the range of 11 to 15 mm. The extracts of *A. marmelos* and *C. longa* showed a fungicidal effect on yeast growth. On the other hand, *C. indicum* extracts exhibited the fungistatic effect (Fig. 1B; Table 2). The essential

oilof *R. officinalis* and *C. citrates* oil exhibited a dose-dependent killing effect on yeast cells (Fig. 1B; Table 2). The plants extracts showing strong anti-proliferative activity was subjected to phytochemical screening to analyse the constituents present. As shown in Table 2, terpenoids, steroids and flavanoids were present in all the plants tested. The presence of tannins was confirmed in all the plant extracts, except *R. officinalis* essential oil. The alkaloids and saponins were present in *C. citratus* oil, and absent in rest of the plant extracts (Table 2). All the plants were positive for the presence of glycosides, except *C. longa* extract (Table 2). Reducing sugars were present only in *C. longa* extracts, and oils from *C. citratus* and *R. officinalis* (Table 2). The phytochemical analysis revealed the presence of coumarins in *C. citratus* oil (Table 2). Proteins were absent in all the plant extracts. Phenolics were present in all plant extracts, except *C. citratus* oil (Table 2). Based on these results, the phytocompound/s responsible for anti-proliferative activity against yeast cells can be purified by bio-assay guided fractionation.

DISCUSSION

Medicinal and aromatic plants are the largest source of bioactive compounds on Earth that have been used by humans for treating various diseases since ancient times. Plant based products have also played an important role in cancer treatment. Vincristine, vinblastine, and taxol are some examples of plant based drugs being used for cancer therapy. The natural products are easily available, cost effective, and less toxic as compared to the allopathic drugs available for cancer treatment. As a result, extensive screens for plant based medicines have gained impetus world-wide[22]. Himalayan region is very rich in the medicinally important plants. Different plant material of 35 plant species was reported for medicinal value for treating various diseases by folklore of tribal communities of chhota Bhangal, Western Himalayas[23]. The hill state of Himachal Pradesh encompasses a diverse range of medicinal flora, whose potential is not fully explored. *S. cerevisiae* has been used as a system for drug discovery[24], but rarely for screening anti-proliferative compounds[25]. There are only few reports on the use of budding yeast for anti-proliferative activities[26]. Our study represents the first report on screening of medicinal and aromatic plants of Himachal Pradesh, India for anti-proliferative activities against *S. cerevisiae. C. indicum* (leaves), *C. longa* (rhizome), *A. marmelos* (leaves, fruit) and *C. citrates* (oil) showed strongest anti-proliferative effect against yeast growth. There are no reports of any of these plants for anti-proliferative effect on budding yeast, *S. cerevisiae.* However, their antimicrobial effects have been well studied in the related pathogenic yeast, *C. albicans*[27]. Hence, the results of the present study are discussed in comparison to these studies. In our study, ethanolic and acetone extracts of leaves of *A. marmelos* exhibited strong anti-proliferative effect, with fungicidal effect on yeast growth, indicating that its phytocompounds kill the yeast cells. *Candida albicans* was found to be the most susceptible fungus to methanolic fruit extract of *A. marmelos* amongst the tested fungal strains[28]. The petroleum ether extract from the leaves of *A. marmelos* exhibited the highest antifungal efficacy against *C. albicans* and other tested fungal species[29]. In our study, ethanolic extracts of *C. indicum* leaves, but not its essential oil, exhibited strong anti-proliferative effect with fungistatic effect on yeast growth (Table 1; data not shown). Pradhan *et al*. (2011) reported that essential oils from *C. indicum* showed maximum antifungal activity at 1% v/v against *Candida albicans* (zone of inhibition of 18.0 mm)[30]. In the present study, ethanolic extracts of *C. longa* exhibited strong fungicidal anti-proliferative effect on yeast growth. Curcuminoids from ethyl acetate extract of *C. longa* rhizomes were found to be antifungal against *Candida albicans*, *C. kruseii* and *C. parapsilosis*[31 32]. Curcumin, a compound isolated from *C. longa*, caused induction of apoptotic cell death and arrested human bladder cancer cells in G2/M phase[33]. However, in the present study, purified curcumin failed to have significant anti-proliferative effect on yeast cells. *C. longa* is documented for anti-inflammatory[34], anticancer, antioxidant and hepato-protective effects[35]. In our study, essential oil of *C. citratus* leaves exhibited strong

anti-proliferative activity, with fungicidal effect on yeast growth. Citral, 3,7-dimethyl-2,6-octadienal, a key component of the essential oil of *C. citratus* was observed to induce apoptosis in acute promyelocytic leukemia cell line (NB4)[36]. In the present study, essential oil of *R. officinalis* leaves showed moderate anti-proliferative effects with fungicidal effect on yeast growth. Antimicrobial activity of the essential oil, and methanolic extract of *R. officinalis* against *S. cerevisiae* and *Candida krusei*[37] was reported by Tavassoli (2011). The anti-proliferative effect of the polyphenols, carnesol and carnosic acid present in *R. officinalis* was reported against colorectal cancer cells[38].

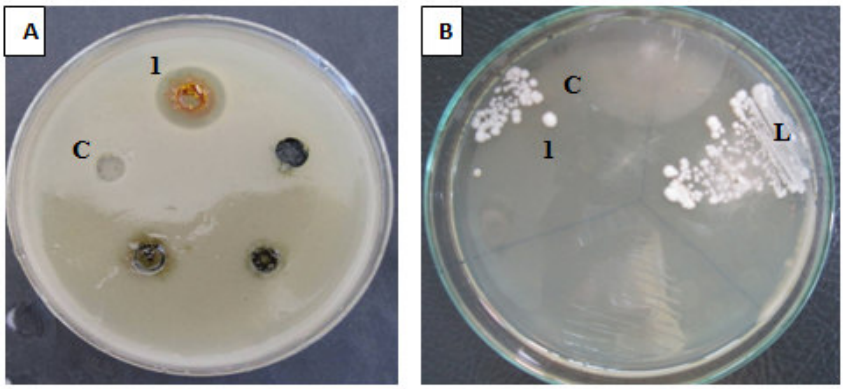

Figure 1

Antiproliferative assay of ethanolic plants extracts against S. cerevisiae. A. A representative result of agar well diffusion assay to test the anti-proliferative effects of ethanolic extract of Curcuma longa. C: solvent control (ethanol); 1-ethanolic extract ofCurcuma longa. The lawn of yeast growth and the zone of clearance of yeast growth caused by the ethanolic extract are visible around the well labeled 1.B.Nature of anti-proliferative effect. The yeast cells from the surrounding the well and the zone of clearance (1) shown in panel A were scraped and streaked onto YPD agar media. L: cells scraped from lawn of yeast cells.

Table 1

Antiproliferative activities of plant extracts against S. cerevisiae. The zone of clearance of yeast growth (mm) produced by the indicated plant extract are listed.

S.No.	Family of the plant used	Name of the medicinal plant / part used	Zone of clearance of yeast growth (mm) ± SE
1.	**Asteraceae**	*Ageratum cornezoids*/ L, F	13.5±0.5
		Chrysanthemum indicum / L	19.5±0.5
2.	**Apocynaceae**	*Catharanthus roseus* / L	Nd
		Nerium indicum / L, F	Nd
		Carissa spinarum / L	Nd
3.	**Lauraceae**	*Cinnamomum camphora* / L	14±0.2
4.	**Zingiberaceae**	*Curcuma longa* / R	26±0.2
		Zingiber officinale / R	Nd
5.	**Ginkgoaceae**	*Ginkgo biloba* / L	14±0.2
6.	**Lamiaceae**	*Salvia officinalis* / Fl	Nd
		Lavendula augustifolia / L	12±0.2
		Mentha citrata / L	Nd
		Vitex nigundo / L	12±0.2
		Rosmarinus officinalis / L*	12±0.2
		Caryopteris divaricata / Fl	12±0.2
		Thymus serpyllum / L	Nd
		Colebrookea oppositifolia / L, F	12±0.2

7.	**Menispermaceae**	*Tinospora cordifolia* / L, Sm	Nd
8.	**Solanaceae**	*Withania somnifera* / L, F	Nd
		Datura metel / L, F	Nd
		Datura stramonium / L, F	Nd
9.	**Acanthaceae**	*Vasica adathoda* / L, F	Nd
10.	**Rhamnaceae**	*Zizyphus mauritiana* / L	Nd
11.	**Convolvulaceae**	*Ipomoea carnea* / L	12±0.2
		Cuscuta reflexa / Sm	Nd
12.	**Rutaceae**	*Murraya koenigii* / L, F	Nd
		Aegle marmelos / L, F	18.5±0.2
		Zanthoxylum armatum / L	Nd
13.	**Meliaceae**	*Azadirachta indica* / L	Nd
14.	**Santalaceae**	*Santalum album* / L	14±0.2
		Osyris arborea / L	Nd
15.	**Malvaceae**	*Bombax ceiba* / Fl	Nd
16.	**Phyllanthaceae**	*Emblica officinalis* / L, F	Nd
17.	**Fabaceae**	*Acacia catechu* / L, B	Nd
		Cassia fistula / L, F	Nd
		Bauhinia variegata / L, F	Nd
		Indigofera tinctoria / Fl	12±0.2
		Abrus precatorius / S	12±0.2
18.	**Musaceae**	*Musa paradisiaca* / L	Nd
19.	**Poaceae**	*Cymbopogon citratus**	19±0.2
20.	**Lythraceae**	*Lawsonia alba* / L	Nd
		Punica granatum / L, F	Nd
21.	**Brassicaceae**	*Raphanus sativus* / L	Nd
22.	**Amaryllidaceae**	*Allium sativum* / Bb	12±0.2
23.	**Ranunculaceae**	*Nigella sativa* / S	Nd
24.	**Hypericaceae**	*Hypericum perforatum* / Fl	Nd
25.	**Urticaceae**	*Utrica dioca* / L	Nd
26.	**Cannabaceae**	*Cannabis sativa* / L	Nd
27.	**Anacardiaceae**	*Rhus cotinus* / L	14±0.2
28.	**Salicaceae**	*Salix herbacea* / L	14±0.2
		Populus Canadensis / L	Nd
29.	**Berberidaceae**	*Podophyllum hexandrum* / S	Nd
		Berberis aristata / L	Nd
30.	**Betulaceae**	*Betula utilis* / L	Nd
31.	**Taxaceae**	*Taxus bacata* / L	Nd
32.	**Combretaceae**	*Terminalia chebula* / F	Nd
33.	**Araceae**	*Colocasia esculenta* / Sm	Nd
34.	**Polygonaceae**	*Rumex acetosa* / L	Nd
35.	**Theaceae**	*Camellia sinensis* / L	Nd
36.	**Colchicaceae**	*Gloriosa superba* / F	Nd
37.	**Asparagaceae**	*Asparagus racemosus* / L	Nd
38.	**Euphorbiaceae**	*Ricinus communis* / L	Nd

Plant parts: L- leaves, F- fruit, Fl-Flower, B- bark, Bb-Bulb, R-Rhizome, S-Seeds, Sm-Stem, Nd: no zone of clearance detected; The zone of clearance values are an average from two independent experiments. SE denotes Standard error. * In case of R. officinalis and C. citratus, essential oil fractions were used in the assay.'

Table 2

Evaluation of the nature of anti-proliferative effect of extracts and essential oils of medicinal plants and their phytochemical screening. Fungicidal indicates no growth of yeast cells, while fungistatic indicates growth of yeast cells when streaked on YPD.

S.no.	Plant name	Part used	Nature of anti-proliferative effect	Test for phytochemicals*										
				1	2	3	4	5	6	7	8	9	10	11
1.	*C. indicum*	Leaves, flower	S	-	+	+	+	+	+	-	-	-	-	+
2.	*C. longa*	Rhizome	C	-	-	+	+	+	+	-	+	-	-	+
3.	*A. marmelos*	Leaves, fruit	C	-	+	+	+	+	+	-	-	-	-	+
4.	*C. citratus*	Oil	C	+	+	+	+	+	+	+	+	+	-	-
5.	*R. officinalis*	Oil	C	-	+	+	+	+	-	-	+	-	-	+

C-fungicidal, S-fungistatic, *1-Alkaloid, 2-Glycosides, 3-Terpenoids, 4-Steroids, 5-Flavanoids, 6-Tannins, 7-Saponins, 8-Reducing Sugars, 9-Coumarins, 10-Proteins, 11-Phenols;- : No reaction in the test; + : Positive reaction in the test for the indicated phytochemical.

CONCLUSION

The study was conducted to screen for anti-proliferative activities of different medicinal and aromatic plants of Himachal Pradesh. The plants showing potent anti-proliferative activity can be used for the isolation of active phyto-compounds. These compounds can further be tested against human cancer cell lines for anticancer therapy, based on the fact that cell cycle genes between *S. cerevisiae* and humans are conserved. Further, our study validates the use of *S. cerevisiae* as an effective and economical tool for screening anti-proliferative activities from medicinal and aromatic plants towards anti-cancer drug discovery.

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