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ANTIFUNGAL ACTIVITY OF ETHANOL AND AQUEOUS EXTRACTS OF SOME MEDICINAL PLANTS AGAINST YEASTS

Botany

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ABSTRACT

BACKGROUND: Many medicinal plants possess antifungal activity due to the presence of secondary metabolites like phenols, phenolic acids, quinones, flavones, flavonoids flavonols, tannins and coumarins, and hydrolytic enzymes which act on membranes of invading microorganisms and cause inhibitory activities against bacteria, fungi and yeasts.
AIM: Evaluation of anti-yeast activity of aqueous and ethanol extract of medicinal plants.
OBJECTIVE: To study the anti-yeast activity of ten important medicinal plants.
METHODS: Ten medicinal plants viz. *Citrus Sinensis* (L) (Orange), *Curcuma longa* L. (Turmeric), *Allium cepa* L. (Onion), *Coriander sativum* L. (Dhania), *Trigonella feonum-graceum* L (Fenugreek), *Bauhinia variegata* (Kachnar), *Oxalis corniculata* L. (Creeping wood sorrel), *Solanum nigrum* Linn (Black nightshade), *Moringa oleifera* L.(Drumstick) and *Azadirachta indica* L. (Neem) were assayed for their antifungal activities against yeasts viz. *Geotrichum candidum, Pichia membranifaciens* and *Yarrowia lipolytica.* Anti-yeast activity was assayed by determining the percentage inhibition of colonial growth by comparing the colony diameter (mm) of poisoned plate (with plant extract) and non poisoned plate.
RESULTS: The results revealed that aqueous and ethanol extract of selected medicinal plants could suppress mycelia growth of these three yeast isolates. In general ethanol extract caused slightly of higher growth inhibition of yeasts than aqueous extract.
CONCLUSIONS: The present findings can form the basis for further investigation to optimize the antifungal activity of herbal extract.

KEYWORDS

Geotrichum candidum, Pichia membranifaciens, Yarrowia lipolytica, Medicinal plants, Aqueous extract, Ethanol extract.

INTRODUCTION

Many herbs possess antimicrobial potential in combination and are considered as alternatives antimicrobial agents[1]. Plants synthesize aromatic secondary metabolites like phenols, phenolic acids, quinones, flavones, flavonoids flavonols, tannins and coumarins[2]. These secondary metabolites and hydrolytic enzymes viz. glucanases and chitinases act specifically on membranes of invading microorganisms[4,5]and cause inhibitory properties against bacteria, fungi and insects[3].

The antifungal activity of medicinal plants has been reported by several workers[6-26]. There is little evidence on the antifungal properties of the medicinal plants and hence present investigation was undertaken.

MATERIALS AND METHODS

Ten medicinal plants viz. *Citrus Sinensis* (L) (Orange), *Curcuma longa* L. (Turmeric), *Allium cepa* L. (Onion), *Coriander sativum* L. (Dhania), *Trigonella feonum-graceum* L (Fenugreek), *Bauhinia variegate* (Kachnar), *Oxalis corniculata* L.(Creeping wood sorrel), *Solanum nigrum* Linn (Black nightshade), *Moringa oleifera* L.(Drumstick) and *Azadirachta indica* (L.) (Neem), were assayed for their antifungal activities against yeasts. The different parts of these plants viz. fruits (peel and juice) of orange, rhizome of turmeric, bulb of onion, leaves of dhania, fenugreek and kachnar, drumstick and neem were used for the preparation of ethanol and aqueous extracts.

Preparation of Extracts

The plant samples were surface sterilization with 5% sodium hypochlorite solution and then dried in shade for 48 hours at ambient temperature. The plant parts were then crushed to fine powder in electric grinder. The aqueous and ethanol crude extracts were prepared from their dried powder. For this purpose twenty five grams (25g) of powder sample was mixed in 100 ml of distilled water and 70% ethanol separately and homogenized in a blender. The mixture was kept undisturbed at room temperature for 24 hrs in sterile flask covered by aluminum foil to avoid 'evaporation. The homogenates obtained were first squeezed out in a muslin fabric square and then filtered through Whatman filter paper (3 mm diameter). After filtration, the extract was evaporated in water bath until 25 ml extract was left in a container. Ethanol and aqueous extracts thus obtained were immediately evaluated for antifungal activities.

Isolation of Yeasts

Yeasts were isolated from spoiled chapatti in YES media consisted of Yeast extract (5g/L), Dextrose (30g/L), Adenine (0.05g/L), Histidine (0.05g/L), Leucine (0.05g/L), Lysine (0.05g/L), Uracil (0.05g/L), Difco bacto agar (20.0 g/L) and Distilled water (1L), and incubated at ambient temperature 25 ± 2^{0}C. Antibiotics viz. Chloramphenicol, Streptopenicillin (50 mg/l) were added to media to inhibit bacterial and fungal growth. Three species of yeast viz. *Geotrichum candidum, Pichia membranifaciens* and *Yarrowia lipolytica* were isolated spoiled chapatti.

Screening of plant extracts for their anti-yeast activities

The anti-yeast activity of aqueous and ethanol extracts was assayed against three isolates viz. *Geotrichum candidum, Pichia membranifaciens* and *Yarrowia lipolytica.*

Five ml of plant extracts from their stock solution was dispensed into 15 ml of molten SDA medium (Sabouraud Dextrose Agar) and poured in 90 mm diameter sterile Petri plates, and swirled to achieve a uniform mixture and allowed them to solidify at room temperature.

Preparation of Inoculums

At least three well isolated colonies of the same type from a culture agar plate were selected. Sterile cork borer (6mm) were used to cut each isolate culture which were at least 5-7 days old. Mycelial disc of each isolate was inoculated into separate plate in three replicates .The plates were then incubated at 25 ± 2^{0}C for 10 days period .Two control sets were set up without extract and preservative (negative control) and other one with chemical preservative (positive control). Colony diameter was recorded by measuring the two opposite circumference of the colony growth. Percentage inhibition of colonial growth was evaluated by comparing the colony diameter of poisoned plate (with plant extract) and non poisoned plate and calculated using the formula given below:

$$GI\ (\%) = \frac{CGc - CGt}{CGc} \times 100$$

GI= Growth inhibition; CGc= colony growth in control; CGt= Colony growth in treatment

All the experiments were conducted in replicates of three and data was recorded as mean value ± SE and Critical difference at 5% level. The results obtained have been presented in Table-1.

Table-1: Antifungal activity of aqueous and ethanolic extract of ten medicinal plants on growth(mm) and per cent inhibition of *Geotrichum candidum, Pichia membranifaciens and Yarrowia lipolytica*

(Colony growth in mm of average of the three replicates ± SE)

Plant sample	Plant parts used in solvents with control		*Geotrichum candidum*		*Pichia membranifaciens*		*Yarrowia lipolytica*	
			Mycelial growth (mm)	% inhibition	Mycelial growth (mm)	% inhibition	Mycelial growth (mm)	% inhibition
	Negative control		90 **±1.15**	0	90 **±1.12**	0	90 **±1.16**	0
	Positive control		0	100	0	100	0	100
Citrus sinensis	Aqueous	Peel	67.83**±1.17**	24.63 **±1.19**	73.00**±2.15**	18.88 **±1.12**	70.50**±3.05**	21.66± **0.65**
		Juice	60.33**±1.25**	32.96 **±1.18**	70.13**±2.35**	22.07 **±1.18**	61.83**±3.11**	31.3**±0.17**
	Ethanolic	Peel	61.16**±2.12**	32.04 **±1.15**	67.67**±2.17**	24.81 **±1.19**	64.00**±2.65**	28.88± **0.2 5**
		Juice	58.67**±1.65**	34.81 **±1.13**	59.13**±1.65**	34.23 **±1.26**	60.00**±2.39**	33.33± **0.37**
Curcuma longa (rhizome)	Aqueous		-	-	-	-	-	-
	Ethanolic		71.5±2.15	20.6± 0.75	74.4±2.35	17.36 ±0.85	56.67±1.16	37.03± 1.16
Allium cepa (bulb)	Aqueous		-	-	-	-	-	-
	Ethanolic		77.7±2.45	13.6± 0.86	81.2±2.75	9.8±0.77	67.80±1.78	24.67± 1.18
Coriander sativum (leaf and stem)	Aqueous		59.67±1.25	33.70 ±1.15	60.16±1.85	33.15 ±1.05	65.80±1.65	26.88± 1.07
	Ethanolic		38.3±1.08	57.44 ±1.26	16.3±1.26	81.88 ±2.36	31.33±1.08	65.18± 1.78
Trigonella foenum-graceum (leaf and stem)	Aqueous		-	-	-	-	-	-
	Ethanolic		62.3±2.16	30.77 ±1.45	64.8±1.76	28.0± 1.08	70.25±2.13	21.94± 0.95
Bouhinia variegata (kachnar)	Aqueous		51.33±0.85	42.96 ±1.21	16.67±1.09	81.47 ±1.71	47.67±1.41	47.03± 1.61
	Ethanolic		6.33±0.55	92.96 ±2.05	5.67±0.71	93.70 ±1.85	8.6±1.06	90.44± 2.61
Oxalis corniculata	Aqueous		30.67±1.26	65.92 ±1.32	28.0±1.32	68.88 ±1.76	40.3±1.61	55.2±1.46
	Ethanolic		8.30±1.45	90.77 ±2.26	11.33±1.12	87.41 ±2.06	8.0±0.61	91.11± 2.14
Solanum nigrum	Aqueous		28.8±1.06	68.0± 2.08	30.16±0.78	66.48 ±1.35	45.67±1.41	49.25± 1.31
	Ethanolic		10.67±0.65	88.14 ±2.35	7.67±0.68	91.47 ±2.15	12.0±0.75	86.66± 1.85
Moringa oleifera (leaf)	Aqueous		61.20±1.08	32.00 ±0.66	58.67±1.16	34.81 ±0.68	62.25±1.30	30.83± 0.92
	Ethanolic		34.20±0.85	62.00 ±1.06	38.30±1.81	57.44 ±1.06	40.23±1.21	55.30± 1.51
Azadirachta indica (leaf)	Aqueous		28.67±0.75	68.14 ±1.54	33.67±1.35	62.58 ±1.62	36.60±1.06	59.33± 1.55
	Ethanolic		14.10±0.61	84.33 ±2.08	13.33±0.68	85.18 ±2.08	16.00±0.68	82.22± 2.06

=Noinhibition

Results

In vitro evaluation of anti-yeast activity of aqueous and ethanol extract of fruit peels and juice of orange on mycelial growth (mm) and per cent inhibition of *Geotrichum candidum, Pichia membranifaciens* and *Yerrowia lipolytica* was undertaken. The results (Table-1) revealed that aqueous and ethanol extract of peel and juice of *Citrus sinensis* could suppress mycelia growth of these three yeast isolates. In general ethanol extract of both peel and juice resulted slightly of higher than aqueous extract. Ethanol juice extract of orange inhibited 34.81, 34.23 and 33.33 per cent of these three test microorganisms.

The aqueous extracts of turmeric, onion and fenugreek had no effect on suppression of mycelial of growth of *G. candidum, P. membranifaciens* and *Y. Lipolytica.* The ethanolic extract caused 20.6%, 17.36% and 37.03% growth inhibition of these three yeasts respectively. The aqueous and ethanolic extracst of dhania caused 33.70% and 57.44%, 33.15% and 81.88%, and 26.88% and 65.18% growth inhibition of *G. candidum, P. membranifaciens* and *Y. lipolytica* respectively. The ethanol extract of this plant caused comparatively more growth inhibition.

Critical examination of the data presented in table indicated that radial growth of all these test yeasts was inhibited irrespective of aqueous and ethanol extracts of all the ten medicinal plants with variable magnitude. The per cent inhibition was always higher in ethanol extract irrespective of test plants as compared to aqueous extract. Ethanol extract of Kachnar in general inhibited 92.96, 93.70 and 90.44 per cent growth of *G. candidum, P. membranifaciens* and *Y. lipolytica* respectively. Similarly, ethanolic extract of wood sorrel inhibited 90.77, 87.41 and 91.18, 87.11 respectively. Ethanol extract of black nightshade inhibited 88.14, 91.87 and 26.66 per cent growth of *G. candidum*, *P. membranifciens* and *Y. lipolytica* respectively.

The results clearly revealed that both aqueous and ethanol extracts of drum stick and neem had the potentiality to suppress growth of the test yeasts. But the extent of growth suppression was always higher in ethanol extract than in aqueous extract. The ethanol extract of Neem responded the best by exhibiting 84.33, 85.18 and 82.22 per cent inhibition in case of *G. candidum, P. membranifaciens* and *Y.lipolytica* respectively. Similarly, the ethanolic extract of Drumstick responded the least recording 62.0, 57.44 and 55.30 in this respect.

DISCUSSION

The antimicrobial activity of medicinal plants against bacteria, fungi and yeasts has been demonstrated by several workers and observed somewhat similar results. The present findings are in conformity with the work of Ehigbai *et al*[27] who investigated the antimicrobial and antioxidant activities of different citrus juice concentrates. Donatus *et al*[28] have investigated the antifungal activity of extracts citrus peel against *Fusarium oxysporum* of Okra plant (*Hibiscus esculentus*). Ashok *et al*[28] and Rajeshwari[29] also studied the antimicrobial activity of orange against *Eschericia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Aspergillus flavus, A. niger, A. parasiticus, Fusarium oxysporum* and *F. verticilloides* and found more or less similar antifungal activity.

Ram Kumar Pundir and Pranay Jain[30] have studied the antimicrobial activity of Black Pepper (*Piper nigrum*) and Turmeric (*Curcuma longa*) extracts and found strong antifungal activity against *Rhizopus stolonifer* and *Mucor* sp. Bhavya Trivedy and Padma Singh[31] have studied the antifungal activity of Cymbopogon citrates (Lemon grass) leaves extract against *Aspergillus flavus* and *Mucor* sp. Sathish Dharani *et al*[32] evaluated the antimicrobial activity of ethanol extract of *Moringa oleifera* and found potent antifungal activity against *Aspergillus niger* and *Aspergillus terreus*. Cecily Rosemary Latha *et al*[33] have investigated the antimicrobial efficacy of *Azadirachta indica* leaf extracts against *Aspergillus niger*, *Mucor* spp. and *Rhizopus* spp. More or less similar the antifungal activity of medicinal plants has been worked out by several workers viz. Neeru Vasudeva and Tanu Sharma[34]; Abd El-Ghany et al[35]; Gupta *et al*[36]; Affhan Shoaib *et al*[37]; Sonali Sewani and Mahajbeen[38]; Jorge *et al*[39]; Suhr and Nielsen[40] etc.

CONCLUSIONS

The present work has identified the anti-yeast activity of aqueous and ethanol extracts against the three yeast isolates. However, further evaluation with the active phytochemicals of these plants is required for definite conclusion of the bioactive compounds responsible for d anti-yeast activity. The present findings can form the basis for further investigation to optimize the antifungal activity of herbal extract.

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