



Determination of Antioxidant Properties and Antimicrobial Activity of Vinyl Phenolic Compounds Extracted from *Saccharomyces cerevisiae* Against Uropathogenic Bacteria

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ABSTRACT

The aim of research study of determining the vinyl phenolic compounds with antioxidant properties and antimicrobial activity using acetone and methanol extracts of *Saccharomyces cerevisiae* (*S. cerevisiae*). The HPLC-UV technique was employed for the identification of the vinyl phenolic compounds and Ferric and Ferrous reducing anti-oxidant power assay along with radical scavenging methods were applied to determine the anti-oxidant properties of the yeast extract. The biochemical tests showed the presence of alkaloids, reducing sugars, steroid, proteins, phenol, cardiac glycosides. Further antimicrobial properties of the yeast extract using Kirby-Bauer disc diffusion method indicates, *E.coli* exhibits the best susceptibility towards the yeast extract. The antimicrobial susceptibility was excellent for all the isolated uropathogens when compared with the standard antibiotics. The metabolites produced by the yeast exhibits vital pharmaceutical important substances such as analgesic, antipyretic, anti-proliferative and antimicrobial properties. This study is a small attempt towards a larger future to serve the mankind with natural remedies.

Keywords: Vinyl Phenolic compound, Antioxidant Properties, Antimicrobial Activity, *Saccharomyces cerevisiae*, Uropathogenic Bacteria.



INTRODUCTION

The major objective of this work is to determine the vinyl phenolic compounds with antioxidant properties of this yeast by acetone and methanol extracts of *S. cerevisiae* and determine the antimicrobial activity^{1,2,3,4}. The HPLC-UV technique was employed for the identification of the vinyl phenolic compounds present in the yeast extract^{5,6,7,8}. The Ferric and Ferrous reducing anti-oxidant power assay along with radical scavenging method using 1,1-diphenyl-2-picryl hydrazyl were applied to determine the anti-oxidant properties of the yeast extract^{4,9,10,12}. The biochemical properties of the yeast extract were determined by different chemical methodologies^{13,14,15}. The antimicrobial properties of the yeast extract was determined by the standard antibiotic sensitivity test methodology designed by the Kirby and Bauer^{7,8,16}. The reactive oxygen species generally gets produced by the cellular metabolism of aerobic bacteria in which the molecular oxygen plays a vital role^{11,17}. They are contributors for the signal transduction of various cellular physiological processes for the human. The diseases such as cardiovascular, Alzheimer, Parkinson, Rheumatoid arthritis, etc. can be caused due to the excess production of reactive oxygen species beyond the threshold antioxidant capacity limit of our biological system which can lead to oxidative stress^{14,18}. The oxidative stress can be minimized by restoring the balance between the oxidant and antioxidant in the human system for the proper function of the physiological system¹⁴. The administration of synthetic antioxidant substances can be a remedy but also results in harmful side effects which can affect the human organs due to its residual effect. In the recent studies the harmful effect of the synthetic chemical compounds on humans was once again debated and hence there is a need for the alternate solution^{7,16}. The alternative solutions should be able to restore the balance between the oxidant and antioxidant in the human system for the proper function of the physiological system and at the same time it should not cause any negative harmful side effects like that of the synthetic compounds¹⁴. The best solution to overcome is to rediscover the nature blessed natural resources used by the ancestors to diagnose various dangerous diseases^{7,8}. The natural products should also be considered only if it is as effective as the synthetic chemical substances⁹. The commonly available brewer's yeast used in

the brewing industries proved to be one of the best natural substance to overcome the synthetic chemical substances and is much more effective^{3,4,9}. This study is intended to study this yeast's potential reactive oxygen capacity. The brewer's yeast employed to our study is *S. cerevisiae*. This yeast possess vinyl phenol compounds 4-vinylphenol and 4-vinylguaiacol in 1:1 ratio which promotes it to be one of the best biopharmaceutical substance^{4,11,13}. The enzymatic activity of *S. cerevisiae* is excellent with substituted cinnamatecarboxy-lyase which converts coumaric and ferulic acids into vinylphenols by its capability of transforming non-oxidative decarboxylation of phenolic acid^{18,19,20}. This endo-cellular activity of this yeast is a potential source which can be used for the prophylaxis of oxidative stress related disorders¹⁴. The metabolites produced by the yeast exhibits vital pharmaceutical important substances such as analgesic, antipyretic, anti-proliferative and cytotoxic antimicrobial properties^{7,8}. The antimicrobial property of the yeast extract was experimented against uropathogenic bacterial infections from isolated specimens collected, purified and identified from the urinary tract infected patients^{7,8,21}.

MATERIALS AND METHODS

Reagents

Chemicals like methanol, DPPH, acetone, FeCl₃, Ninhydrin reagent, Fehlings, Benedicts, Salkowski's reagents were procured from Sigma-Aldrich chemicals (USA). Microbiological selective isolation culture media along with biochemical identification reagents and standard antimicrobial discs were employed. All other chemicals were analytical grade.

Yeast collection and Identification

A purified and isolated standard strain sample of the yeast *S. cerevisiae* from one of the ATCC (American Type Collection Centre) specimen collection centre in India and was used for this experiment. The confirmation identification for the pure culture of the yeast was verified by employing various in vitro fungal identification technique.

Preparation of the yeast extracts

10 g of the dried yeast samples were sequentially extracted with 250 mL of acetone and methanol at 37°C for 24 hours^{1,2}. A rotatory evaporator was employed to dry the concentrated

extract under reduced pressure with the temperature of 40°C and stored in the sterile test tubes at -20°C. The yield of respective yeast extracts were estimated as Yield(%) = (dry weight of extract/dry weight of samples) x100

Identification of the vinyl phenolic compound in the yeast

Based on Huneck and Yoshimura the identification of the vinyl phenolic compounds present in the yeast *S. cerevisiae* were performed by HPLC-UV analytical technique²⁰. The stored dried yeast extracts were dissolved in 500 µL of acetone and analyzed by using Agilent Technologies, 1200 Series HPLC instrument with C18 column 25cm x 4.6mm, 10µm with UV spectrophotometric detector.

Determination of anti-oxidant properties in the yeast

- 1) Based on the Oyaizu Ferric and Ferrous Reducing-Antioxidant Power (FRAP) assay methodology the anti-oxidant properties of yeast extract of *S. cerevisiae* was determined by the reducing powers of the dried yeast extracts by using various concentrations of the extract ranging from 50 to 1000 µg/mL diluted with saline buffer 0.2 M, pH 6.6 & 2.5 mL of ferric chloride (1%) and ferrous solution (1%) respectively and incubated at 50°C for 30 minute. After incubation period 2.5 mL of 10% trichloroacetic acid (TCA) was added to the mixture to stop the reaction. The mixture was centrifugated at 3000 g for 10 min and 2.5 mL distilled water added to 25 mL of the supernatant with 0.5 mL FeCl₃ (0.1%) and then the optical density was observed at 700 nm with an American made UNICO spectrophotometer which determined that the higher absorbance of the reaction mixture which indicates greater reducing power of the yeast. Suitable positive and negative controls were used for the test. The test was repeated for the five times and the mean value was recorded for the accuracy of the results.
- 2) Based on Kosanic *et al.*, radical scavenging methodology using 1,1-diphenyl-2-picrylhydrazyl, the anti-oxidant properties of the yeast extract of *S. cerevisiae* to scavenge DPPH free radicals was estimated by the reduction of the reaction color between

DPPH solution and sample extracts was determined by adding 2 mL of 0.12 mM solution DPPH in methanol to the 1 mL of various concentrations ranging from 50 - 1000 µg/mL of the yeast extract respectively. The mixture was incubated at 37°C for 30 min and the absorbance of the reaction mixture was measured at 517 nm with an American made UNICO spectrophotometer to determine the radical scavenging activity of the yeast extract. Suitable positive and negative controls were used for the test. The test was repeated for the five times and the mean value was recorded for the accuracy of the results.

Bio-chemical analysis of the yeast extract

The biochemical properties of the yeast extract of *S. cerevisiae* was determined by Mayer's test, Fehling's test, Salkowski's test and Ninhydrin test, Ferric Chloride test, Libermann Burchard's Test, Benedict's Test, Keller-kilani test and ammonia test¹³.

Antimicrobial Property of the yeast extract

The antimicrobial activity of the yeast extract was performed against the clinically isolated uropathogens from the urinary tract infected persons.

Isolation and Identification of uropathogenic Specimen

The clinical urinary sample was collected from the suspected urinary tract infected patients and inoculated on the selective media Cystine Lactose Electrolyte Deficient Agar with pH indicator bromothymol blue. The inoculated plates were incubated at 37°C for 24 h and biochemical reaction test were done for all the isolated uropathogens from the isolated colonies by using IMVIC test and results were observed respectively²¹.

Antibiotic Susceptibility testing by Kirby-Bauer Disc Diffusion Method

Based on the standard antibiotic sensitivity methodology developed by Kirby and Bauer the antimicrobial activity of the yeast extract were determined^{7,8,16} by incorporating the standard antibiotic discs coated with the yeast extract on the various Mueller Hinton agar plates inoculated with different uropathogenic samples such as *E.coli*, *Klebsiella Sp.*, *Pseudomonas Sp.* and *Proteus Sp.* isolated from the clinical urine specimens respectively. The inoculated plates impregnated

with the antibiotic disc of yeast extract were incubated for 24 h at 37°C. The zone formation around the disc indicates the susceptibility of the organism and no zone formation indicates the resistance of the organism towards the antibiotic disc of the yeast extract.

RESULTS AND DISCUSSION

The yeast extract results were exemplary for all the tests performed. Based on Huneck and Yoshimura the identification of the vinyl phenolic compounds present in the *S. cerevisiae* were performed by the HPLC-UV analytical technique analysis of acetone and methanol extracts of the yeast extract was used to identify their major vinyl phenolic compounds Figure 1.

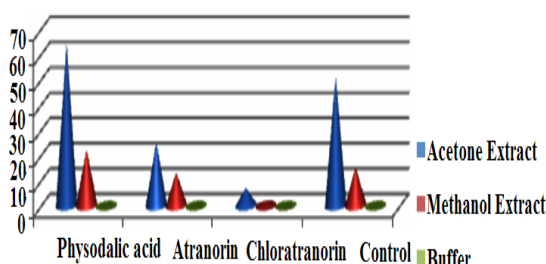


Fig.1. Analysis of a vinyl phenolic compounds present in the *Saccharomyces cerevisiae* (acetone and methanol extracts) by HPLC-UV technique

By comparing the retention times (tR) yeast extract were identified. The main compounds in acetone extract of yeast were physodalic acid (tR = 3.295 ± 0.012 min), atranorin (tR = 8.642 ± 0.020 min) and chloratranorin (tR = 11.918 ± 0.026 min). The most abundant substance in the acetone yeast extract was physodalic acid where as in methanol yeast extract only small amount of physodalic acid was observed in the chromatogram. It is evidenced in the chromatograms of ferric and ferrous reducing power assay of oxidant/antioxidant based on Oyaizu technique as shown in Figure 2.

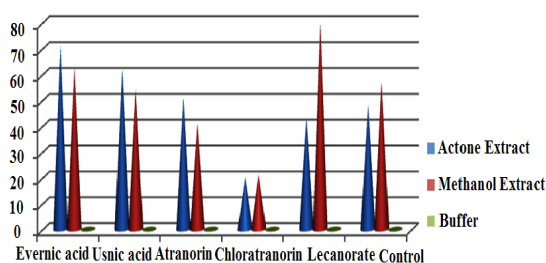


Fig. 2. Chromatograms of ferric and ferrous reducing power assay of oxidant/antioxidant

The chromatogram showed evernic acid, (tR = 3.045 ± 0.029 min), usnic acid (tR = 7.854 ± 0.025 min), atranorin (tR = 8.236 ± 0.028 min) and chloratranorin (tR = 11.554 ± 0.028 min) are present in the acetone extracts. The most abundant compound in acetone extract is evernic acid while in methanol extract also evernic acid and usnic acid detected but the methyl lecanorate (tR = 1.515 ± 0.009 min) is the most abundant compound.

Based on Kosanic *et al.*, radical scavenging methodology using 1,1-diphenyl-2-picryl hydrazyl, the anti-oxidant properties of the yeast extract of *S. cerevisiae* to scavenge DPPH free radicals was estimated by the reduction of the reaction color between DPPH solution and sample extracts was determined results showed a statistically significant data as the antioxidant activity was elevated from 2.01 ± 0.009% to 86.14 ± 0.013% in accordance with the increase of the concentration of the extracts from 50 to 1000 µg/mL of yeast extract Figure 3.

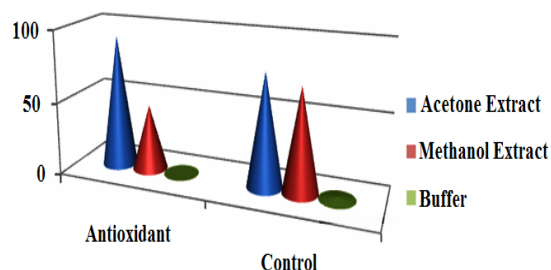


Fig. 3. Radical scavenging methodology using 1,1-diphenyl-2-picryl hydrazyl, the anti-oxidant properties of the yeast extract of *Saccharomyces cerevisiae* to scavenge DPPH free radicals

The acetone yeast extract with 1000 µg/mL showed largest DPPH radical scavenging activity: 86.14 ± 0.013%. According to test results the antioxidant activities obtained from the methanol yeast extracts were obtained but lower than that measured for the acetone yeast extract which showed the highest DPPH radical scavenging activity with an IC₅₀ = 240.220 ± 15.165 µg/mL.

The biochemical properties analysis of the yeast *S. cerevisiae* extract showed the presence of alkaloids, reducing sugar, steroids, protein, phenol, glycosides, cardiac glycosides and amino acids as shown in Table 1.

Table 1: Biochemical properties of yeast

Test	Observation	Result	Interpretation
Mayer's test	Formation of a creamy substance	Positive	Presence of alkaloids.
Fehling's test	Brick red color formation	Positive	Presence of reducing sugar
Salkowski's test	Reddish brown color formation	Positive	Presence of steroids
Ninhydrin test	Violet color formation	Positive	Presence of protein
Ferric chloride test	Blue black coloration	Positive	Presence of Phenol
Libermann Burchard's test	Violet to bluish green color formation	Positive	Presence of Glycosides
Benedict's test	Formation of orange red precipitate	Positive	Presence of reducing sugar
Keller-kilani test	Brown ring at the interface	Positive	Presence of Cardiac glycosides
Ammonia test	Yellowish color formation	Positive	Presence of Amino Acids

Further, collected specimens from the urinary tract infected persons were isolated by culturing on a selective media Cystine Lactose Electrolyte Deficient Agar with pH indicator Bromothymol blue showed Yellow lactose fermenting colonies for the *Klebsiella sp.*

and *E.coli* whereas blue non lactose fermenting colonies for the *Proteus sp.* and *Pseudomonas sp.* The isolated organisms were confirmed using biochemical reactions of IMVIC and the result observed were tabulated (Table 2) and confirmed the identification of the pathogens.

Table 2: biochemical assessment of the isolated uropathogens

Organism	Indole	Methyl red	Voges proskauer	Citrate	Urease	Oxidase
<i>E.Coli</i>	pink ring Positive	red color positive	yellow color negative	green color negative	yellow color negative	no purple color negative
<i>Klebsiella sp.</i>	yellow ring Negative	yellow color negative	red color positive	blue color positive	pink color positive	no purple color negative
<i>Pseudomonas sp.</i>	yellow ring Negative	yellow color negative	yellow color negative	blue color positive	yellow color negative	purple color positive
<i>Proteus sp.</i>	yellow ring Negative	red color positive	yellow color negative	blue color positive	pink color positive	no purple color negative

Then using the Kirby-Bauer disc diffusion method the antimicrobial susceptibility of the prepared yeast extract antibiotic disc were tested against the isolated uropathogens and the results were exemplary. *E.coli* shown the best susceptibility towards the yeast extract while the *Pseudomonas*

sp. show the least susceptibility. The *Klebsiella sp.* and *Proteus sp.* shown a moderate susceptibility with the yeast extract antibiotic disc. The antimicrobial susceptibility was excellent for all the isolated uropathogens when compared with the standard antibiotics (Table 3).

Table 3: Antimicrobial susceptibility comparison of yeast extract with standard antibiotics for urinary tract infections

Antimicrobics	<i>E.coli</i>	<i>Klebsiella sp.</i>	<i>Pseudomonas sp.</i>	<i>Proteus sp.</i>
Yeast Extract	Sensitive 25mm	Sensitive 23mm	Sensitive 19mm	Sensitive 21mm
Trimethoprim	Sensitive 21mm	Intermediate 15mm	Resistant 2mm	Intermediate 14mm
Sulfamethoxazole	Sensitive 18mm	Sensitive 24mm	Resistant 4mm	Sensitive 19mm
Fosfomycin	Sensitive 21mm	Sensitive 22mm	Sensitive 21mm	Sensitive 18mm
Nitrofurantoin	Sensitive 26mm	Sensitive 21mm	Sensitive 20mm	Sensitive 18mm
Azithromycin	Sensitive 24mm	Sensitive 19mm	Sensitive 22mm	Sensitive 22mm
Ceftriaxone	Sensitive 19mm	Sensitive 24mm	Sensitive 20mm	Sensitive 24mm
Cephalexin	Sensitive 20mm	Sensitive 21mm	Sensitive 18mm	Sensitive 21mm
Levofloxacin	Intermediate 16mm	Resistant 8mm	Resistant 2mm	Resistant 6mm
Ciprofloxacin	Sensitive 22mm	Sensitive 19mm	Intermediate 14mm	Intermediate 13mm

CONCLUSION

The antioxidant properties along with the vinyl phenolic compounds of the yeast *Saccharomyces cerevisiae* shows that this solated yeast extract can be used as a potential anti synthetic substance for the treatment of many diseases not only just as an antibiotic but can also help to balance between the oxidant and antioxidant level in the body for the proper function of the physiological system in maintaining the proper body functions as it contains many essential biochemical nutrients required for our body to produce

reactive oxygen species to eradicate many diseases. This study is a small attempt towards a larger future to serve the mankind with natural remedies.

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Conflicts of interest

No conflict of interest.

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