Phytochemical analysis and *in vitro* antioxidant, antimicrobial, anti-inflammatory and cytotoxicity activities of wood rotting fungi, *Trametes ochracea*

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ABSTRACT

Objective: The present research was aimed to identify the metabolites in the methaolic and hexane extract of *Trametes ochracea* and evaluated these extracts to know their *in vitro* biological activities. **Materials and methods:** Two solvent extracts of *T. ochracea* was subjected to phytochemical analysis. The two solvent extracts were subjected to in vitro biological activity viz., antioxidant, anti-inflammatory and cytoxicity assays. **Results:** The methanol extract yielded important phytochemicals viz., saponins, flavonoids, alkaloids, steroids, phenols and tannins compared to hexane. The methanolic extract has shown strong antioxidant activity in all tested methods. The methanol extract was effectively inhibited the heat induced hemolysis, antilipoxygenase activity and also stabilized the membrane, avoided the membrane denaturation, proteinase and xanthine oxidase inhibition.The onion root meristametic cells were inhibited due to toxicity of methanol extract by possessing various cellular abnormalities in various stages of actively growing cells. The yeast cells were dead due to toxicity of methanol extract by possessing cell necrosis and also fragmented the cell DNA. **Conclusion:** The obtained results clearly indicates that *Trametes ochracea* methanol extract is having potent phytochemicals, which plays important role in antioxidant, anti-inflammatory, cytotoxicity assays. The further research is needed to identify the exact mechanism is by action of one or combination of active phytochemicals.

Key words: *Trametes ochracea*, Phytochemicals, Antioxidant, Anti-inflammatory, Cytotoxicity.

INTRODUCTION

During the process of oxygen utilization in a normal physiological and catabolic process, approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals (ROS) like superoxide anions (O^{2-}), hydroxyl (.OH) nitric oxide (NO), which damage cellular components causing tissue injury through covalent binding.[1,2] Free radicals have been implicated in causation of diseases such as diabetes, inflammation, cancer, neurodegenerative disorders, atherosclerosis, liver cirrhosis, nephrotoxicity etc.[3] It has been suggested that fruits, vegetables, plants are the main source of antioxidant in the diet. Natural antioxidants may have free-radical scavengers, reducing agents, complexes of pro-oxidant metals, quenchers of singlet oxygen etc. Recently interest has been increased considerably in finding natural occurring antioxidants for use in foods or medicinal products to replace synthetic antioxidants, which are being restricted due to their adverse reaction such as carcinogenicity. Antioxidant constituents from natural resources possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance.[4] Free radicals which have one or more unpraired electrons (superoxide, hydroxyl, peroxyl) are produced in normal or pathological cell

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metabolism and the compounds that can scavenge free radicals have great potential in ameliorating the diseases and pathological cells.[5-7] Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species. Free radicals or Reactive Oxygen Species (ROS) are produced *in vivo* from various biochemical reactions and also from the respiratory chain as a result occasional challenges. These free radicals are the main culprits in lipid peroxidation. Plants congaing bioactive compounds have been reported to possess strong there is excessive activation of phagocytes, production of O^{2-}, OH radicals as well as non free radicals species (H_20_2)[8] which can harm severely tissues either by powerful direct oxidizing action or indirect with hydrogen peroxide and –OH radical formed from O2- which initiates lipid peroxidation resulting in membrane destruction. Tissue damage then provokes inflammatory response by production of mediators and chemotactic factors.[9] The reactive oxygen species are also known to activate matrix metello proteinase damage seen in various arthritic tissues.[10] The literature survey is giving the importance of plants and their antioxidant properties[11] plant active compounds especially phenolic and flavonoid compounds have proved as potent antioxidant and free radical scavenger.[12]

Basidiomycetes are an useful natural products with various biological activities and they are called as medicinal mushrooms. They may be edible or non-edible mushroom species.[13-15] *Trametes ochracea* is a white coloured wood rotting fungi, used to produce laccase enzyme.[16]

The present investigation was aimed to identify phytochemicals in different solvent extracts and this extract was evaluated for different *in vitro* biological activities (antioxidant, anti-inflammatory, cytotoxicity).

MATERIALS AND METHODS

Collection of fungal material and extract preparation

The white rot fungi grown on wood were collected near our college campus (Shridevi Institute of Engineering & Technology, Sira Road, Tumakuru, Karnataka, India). The collected fungus was identified as *Trametes ochracea* based on colour, morphology and spore by using fungal manuals. Identification of the different phytochemical from *T. ochracea* was carried out using methanol and hexane solvent at 5 g/15 ml (w/v) separately for 2 days with a shaking attachment. The sample was air dried at room temperature (26 ± 2^{0}C) for 4 weeks to get consistent weight. The dried parts were later ground to powder. The extract was lyophilized under 5 µm Hg pressure and stored at -20^{0}C. The experiments were carried out using an appropriate amount of lyophilized material.

Phytochemical analysis

Phytochemical analysis was carried out for saponins, flavonoids, cardiac glycosides, terpenoids, steroids, tannins, phenol, anthroquinone, alkaloids and tannins was performed as described by the authors.[17-19] Wagner's and Heger's reagents were used for alkaloid foam test for saponins, Mg-HCl and Zn-HCl for flavonoids, Keller-Killani test for cardiac glycosides, Salkonoski test for terpenoids, acetic anhydride and sulphuric acid for steroids, chloride and gelatin for tannins, ferric chloride for phenol test. All these experiments were carried out for methanol and hexane extracts of *T.ochracea*.

Determination of antioxidant activity

In order to investigate the antioxidant properties of the examined extracts, ferric ion reducing antioxidant power (FRAP), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS assays.

DPPH radical scavenging assay

The free radical scavenging activities of each extract were measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). Briefly, extract concentration of (0.1-20 mg/ml) in methanol (4 ml) was mixed with 1 ml of methanol solution containing DPPH (Sigma) radicals of 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min in the dark and the absorbance was measured at 517 nm against a blank.[20] EC_{50} value (mg/ml) is the effective concentration at which DPPH radicals were scavenged by 50% and the value was obtained by interpolation from linear regression analysis. BHT was used as a standard for the comparison. The capability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH\ scavenging\ effect\ (\%) = [\{A_0 - A_1\}A_0\,] \times 100,$$

Where, A_0 is the absorbance of the control reaction and A_1 the absorbance in the presence of the sample. The extract concentration providing 50% inhibition (EC_{50}) was calculated was obtained by interpolation from linear regression analysis.

ABTS radical scavenging activity

The two stock solutions included 7.4 mM

2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2.6 mM potassium persulphate was prepared.[21] The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in dark. The solution was diluted by mixing with 1 ml ABTS solution prepared using 50 ml of methanol, in order to obtain absorbance 1.1 ± 0.02 units at 734 nm. Samples (1.5 ml) were mixed with 2.850 ml of ABTS solution and the mixture was left at room temperature for 2 h in dark. The capability to scavenge the ABTS radical was calculated using the following equation:

$$ABTS\ scavenging\ effect\ (\%) = [\{A_0 - A_1\}A_0] \times 100,$$

Where, A_0 is the absorbance of the control reaction and A_1 the absorbance in the presence of the sample.

The extract concentration providing 50% inhibition (EC_{50}) was calculated was obtained by interpolation from linear regression analysis.

FRAP assay

FRAP reagents were freshly prepared by mixing 25 mL acetate buffer (300 mM, pH 3.6), 2.5 mL 2,4,6-Tris (2-pyridyl)-S-triazine (TPTZ) solution (10 mM TPTZ in 40 mM/L HCl) and 2.5 mL $FeCl_3$ (20 mM) water solution. Each sample (150 µL) (0.5 mg/mL) dissolved in methanol was added in 4.5 mL of freshly prepared FRAP reagent and stirred and after 5 min, absorbance was measured at 593nm, using FRAP work solution as blank.[22] A calibration curve of ferrous sulfate (100-1000 µmol/L) was used and results were expressed in µmol Fe2+/mg dry weight extract. The relative activity of the samples was compared to L-ascorbic acid.

Determination of total phenolic content

Total Phenolic Content (TPC) in two solvent extracts of wood rotting fungi was determined,[23] using Folin-Ciocalteu's colorimetric method. To 5 ml of 0.3% HCl in methanol/deionised water (60:40, v/v), 100 mg of the ethanol extract was added. From the resulting mixture (100 µl) was added to 2 ml of 2% aqueous sodium carbonate. The mixture was incubated for 2 min. To that 100 µl of 50% Folin-Ciocalteu's reagent was added and incubated for 30 min, absorbance was measured at 750 nm against blank. The content of total phenol was calculated on the basis of the calibration curve of gallic acid and the results were expressed as mg of gallic acid equivalents (GAEs) per g of extract.[24]

Flavonoid determination

Total flavonoid was determined.[25] The fungal extract (250 µl) was mixed with distilled water (1.25 ml) and $NaNO_2$ solution (5%, 75 µl). After 5 min the $AlCl_3$ H_2O solution (10%, 150 µl) was added. After 6 min, NaOH (1M, 500 µl) and distilled water (275 µl) was added to the mixture. The solution was mixed well and the intensity of the pink color was measured at 510 nm against blank. The content of flavonoid was calculated on the basis of the calibration curve of quercetin and the results were expressed as mg of quercetin equivalents per g of extract.

In vitro anti-inflammatory activity

Inhibition of albumin denaturation

Methods of Mizushima and Kobayashi[26,27] were followed with minor modifications. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using a small amount at 37^{0}C HCl. The sample extracts were incubated at 37^{0}C for 20 min and then heated to 51^{0}C for 20 min after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows,

$$\%\ inhibition = [\{Abs\ control - Abs\ sample\}/Abs\ control] \times 100,$$

Where, Abs control is the absorbance without sample, Abs sample is the absorbance of sample extract/standard.

Membrane stabilization test

Preparation of red blood cells (RBCs) suspension

Fresh whole human blood (10 ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline.[28,27]

Heat induced hemolytic activity

The reaction mixture (2 ml) consisted of 1 ml of test sample solutions and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water-bath at 56^{0}C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture

was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatant was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by the formula mentioned above.[27,29]

Protein inhibitory action

The test was performed according to the modified method[27,30] The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml of 20 mM Tris-HCl buffer (pH7.4) and 1ml test sample of different concentrations of different solvents. The reaction mixture was incubated at 37^{0}C for 5 min and then 1ml of 0.8% (W/V) casein was added. The mixture was inhibited for an additional 20 min, 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated.

Anti-lipoxygenase activity

Anti-lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme.[29] Test samples were dissolved in 0.25 ml of 2 M borate buffer pH 9.0 and added 0.25 ml of lipoxidase enzyme solution (20,000 U/ml) and incubated for 5 min at 25^{0}C. After which, 1.0 ml of lenoleic acid solution (0.6 mM) was added, mixed well and absorbance was measured at 234 nm. Indomethcin was used as reference standard. The percent inhibition was calculated from the following equation,

$$\% \text{ inhibition} = [\{Abs \text{ control} - Abs \text{ sample}\}/Abs \text{ control}] \times 100,$$

A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

Xanthine oxidase assay

Xanthine oxidase activity was assayed spectrophotometrically at 300 nm as described.[31] Briefly, the reaction mixture consisting of 500 µl of solution A, (0.1M phosphate buffer containing 0.4 mM xanthine and 0.24 mM NBT), 500 µl of solution B (0.1 M phosphate buffer containing 0.0449 units/ml xanthine oxidase) and 50 µl of a 10% of each solvent extracts were incubated in a cuvette at 37^{0}C for 20 min. The enzyme activity was expressed as the increment in absorption at 300 nm per unit time.

Acetylcholinesterase (AChE) inhibitory activity

The AChE inhibitory assay and inhibition kinetics analysis was conducted according to the protocol[32] with some modifications. The assay mixture consisted of 200 µL of Tris-HCl 50 mM pH 8.0, 0.1% BSA buffer, 100 µL of extracts or fractions solution (final concentration: 100 µg mL-1) was dissolved in buffer-MeOH (10%) and 100 µL of AChE (0.22 U mL-1). The mixture was incubated at room temperature for 2 min before the addition of 500 µL of DTNB (5,5 Vdithiobis [2-nitrobenzoic acid] (3 mM) and 100 µL of substrate acetylthiocholine iodide (ATCI) (15 mM). The developing yellow color was measured at 405 nm after 4 min. Galantamine was used as positive control at a final concentration of 0.2 µg mL-1 in the assay mixture.

AChE inhibitory activity was expressed as percent inhibition of AChE, calculated as (1-B/A) X 100, where A is the change in absorbance of the assay without the plant extract (Δ abs. with enzyme- Δabs. without enzyme) and B is the change in absorbance of the assay with the plant extract (Δ abs. with enzyme - Δ abs. without enzyme).

Antimitotic activity

Method adopted[33] was used for determination of antimitotic activity using *Allium cepa* root with slight modification. *A. cepa* were collected from Tumkur vegetable market. *Allium cepa* bulbs were sprouted in water for 24 h at room temperature. The uniform root tips of *A. cepa* were selected for the study. These roots were dipped in the extract (10 mg/mL and 5 mg/mL) for 48 hours. Water was used for dilution and lapachol was used as a standard for study. After 48h, the root tips were fixed in the fixing solution of acetic acid and alcohol (1:3). Squash preparation was made by staining with acetocarmine stain. Morphology and the number of the cells were observed under microscope (40x). In all 350-400 cells were counted and cells manifesting different stages of mitosis i.e., interphase (I) and prophase (P), metaphase (M), anaphase (A) and telophase (T) were recorded. The mitotic index was calculated using the following formula.[33,34]

$$\text{Mitotic index} = [P + M + A + T/ \text{Total cells}] X 100$$

Antiproliferative activity

Evaluation of antiproliferative activities of plant extract was done by yeast *Saccharomyces cerevisiae* model.[33]

Yeast inoculum preparation

The yeast was inoculated with sterilized potato dextrose

Table 1: The yield of different phytochemicals from two solvent extracts of *T. ochracea*

Phytochemicals	Solvent extracts	
	Methanol	Hexane
Proteins	+	+
Carbohydrates	+++	+
Resins	+	+
Saponins	++	-
Flavonoids	++	-
Alkaloids	++	+
Steroids	++	+
Phenols	++	+
Tannins	++	+

+: ++: +++: -:, data based on triplicate result of each sample.

broth and incubated at 37°C for 24 h and it was referred as seeded broth.

Determination of cell viability

Cell viability assay was performed with 2.5 mL of potato dextrose broth and 0.5 mL of yeast inoculum in four separate test tubes. In the first test tube distilled water, in second test tube quercetin (Sigma-Aldrich) as standard (1 mg/mL), in third and fourth test tubes plant extract (10 mg/mL and 5 mg/mL respectively) was added. All tubes were incubated at 37°C for 24 hours. In the above cell suspension, 0.1%methylene blue dye was added in all tubes and they were observed under low power microscope. The number of viable cells, those does not stain and look transparent with oval shape while dead cells get stained and appeared blue in color were counted in 16 chambers of hemocytometer and the average number of cell was calculated. The percentage of cell viability was calculated using the formula.[35]

% Cytotoxicity = No. of dead cells/ No. of viable cell + No. of dead cells X 100

DNA fragmentation assay

DNA fragmentation[36] assay was performed by the method briefly, 0.1 mL of extract mixed with 2.5 mL potato dextrose broth and 0.5 mL of yeast inoculums. Cell suspension was incubated for 24 h at 37°C. DNA was isolated from the treated cell suspension with Tris-EDTA buffer and DNA was electrophoresed.[33]

Statistical analysis

Analysis of variance (ANOVA) was used to determine the significance of difference between treatment groups ($p<0.05$). Means between treatment groups were compared for significance using Duncan's new Multiple Range post test.

RESULTS AND DISCUSSION

The two solvent extracts (methanol and hexane) of *T.ochracea* yielded nine different phytochemicals. Maximum concentrations of phytochemicals were observed in methanol extract. The saponins, flavonoids, alkaloids, steroids, phenols and tannins were present at higher concentration in methanol extract compared with hexane (Table 1). The hexane yielded fewer amounts of the phytochemicals. The antioxidant activities of the both solvent extract were studied by measuring the ability of scavenging DPPH free radicals was compared with standard Butylated Hydroxy Toluene (BHT). The methanol extract have showed higher activity of DPPH followed by heaxane extract. At a concentration of 0.1 mg/ml, the scavenging activity of methanol and hexane extract was reached to (97) and (71) respectively. The DPPH radical scavenging ability of the extracts was less than that of standard, BHT (99) (Figure 1). The study confirms that, *T. ochracea* extracts have the proton donating ability and could be serve as free radical inhibitor or scavenging activity possibly as primary antioxidant. Our results are confirmatory wth the reports[24,37,38] but they used different source such as red algae, endophytic fungi etc.

The two solvent extract of *T. ochracea* reacted with different concentration (100, 200, 400, 800, 1600 µg/ml) of ABTS and reading were measured at 734 nm for observation of reduction of radical cation generated by ABTS+. The methanol extract have showed maximum decoloraion (Figure 2). ABTS assay is considered as an excellent tool for determine the antioxidant activity. The edible basidiomycetes and endophytes assayed against ABTS radical and reported to have scavenging ability these radicals.[39,37]

The reduction ability of Fe (II)/mg was range from 1346.16 to 482.55 by methanol and hexane extract respectively. May be the extracts due to their ability, reduced the TPRZ-Fe(III) to TPTZ-Fe(II) (Figure 3). The hydrogen peroxide activity was more in methanol extracts of *T. ochracea* (Figure 4). The methanol extract value is significantly lower than that of standard ascorbic acid (1648.96). The important role of flavonoid is stabilizing lipid oxidation associated with antioxidant activity. The flavonoid content of methanol and hexane extract was 21.36 and 8.74 µg/ml equivalent. The results are confirmatored.[37]

Anti-inflammatory assay

A cause of inflammation is nothing but denaturation of proteins. The two solvents (methanol and hexane) extract

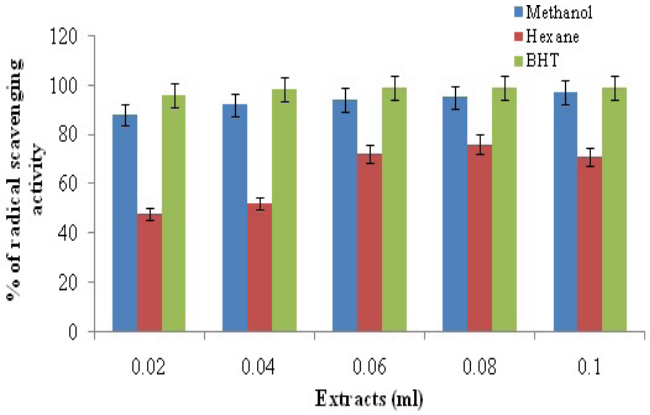

Figure 1: *In vitro* DPPH activities of different solvent extracts of *T. ochracea*

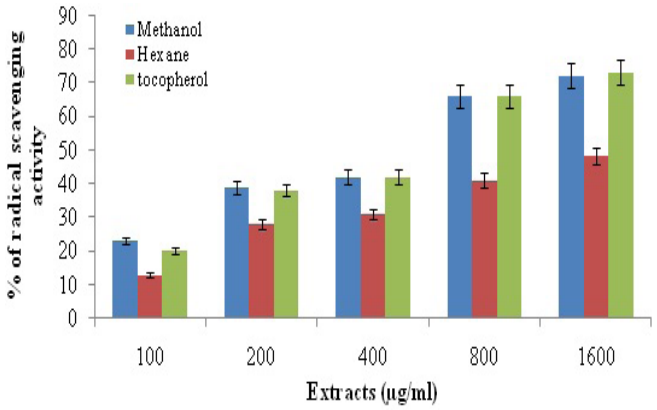

Figure 2: Free radical scavenging activities of *T. ochracea* against ABTS

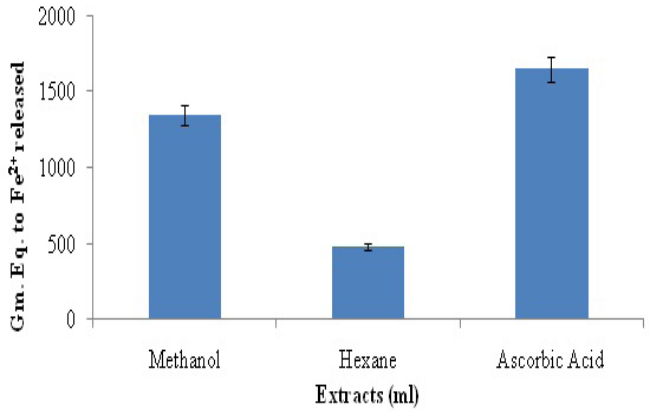

Figure 3: Total antioxidant (FRAP) activies of two solvent extracts of *T. ochracea*

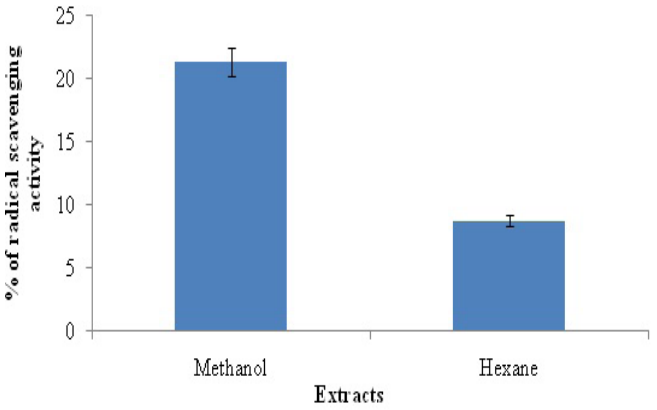

Figure 4: Effect of *T. ochracea* extracts on hydrogen peroxide scavenging activity

Table 2: Effect of different solvent extracts of *T. ochracea* on albumin denaturation, membrane stabilization and proteinase inhibitory activity percentage inhibition

Test Samples	Membrane stabilization	Albumin denaturation	Proteinase inhibition
Methanol	73.45±0.08	88.11±0.16	84.82±0.22
Hexane	44.78±0.13	51.63± 0.06	53.41±0.12
Aspirin (200 µg/ml)	85.92±0.18	75.80.099±0.05	92.83±0.30

of *T. ochracea* was used to study the mechanism of anti-inflammatory activity of membrane stabilization test using RBC membrane. The maximum inhibition was observed from methanol extract (73.45) followed by hexane (44.78). A standard anti-inflammatory drug, Aspirin showed the maximum inhibition of (85.92) at the concentration of 200 µg/ml. The methanol extract was effectively inhibiting the heat induced hemolysis of anti-inflammatory effect. The effect may be due to presence of phytochemicals present in the extracts possibly inhibited the release of lysosomal content of neutrophils as the site of inflammation. The two extracts inhibited the heat induced hemolysis of RBC's at varying degree (Table 2). The maximum inhibition was observed in methanol extract (88.11) compared to hexane (51.63). The methanol extract (84.82) exhibited significantly antiproteinase activity compared with hexane extract (53.41). The maximum proteinase activity was observed from standard drug aspirin (92.83).

Antilipoxynase[29] using linoleic acid as substrate and lipoxidase as enzyme. The maximum activity was observed with methanol extract (57.14) and hexane was showed less activity (34.28) compared with standard indomethacin showed 52.20% inhibition at a concentration of 60 µg/ml (Figure 5).

The first number of requirements for the developments of medicines for treating some diseases is acetylcholinesterase inhibitory activity. The two solvent (methanol and hexane) extracts of *Trametes ochracea* were used for *in vitro* acetylcholine inhibitory activity at a concentration of 100 µg/ml and in the assay mixture galanthamine used as

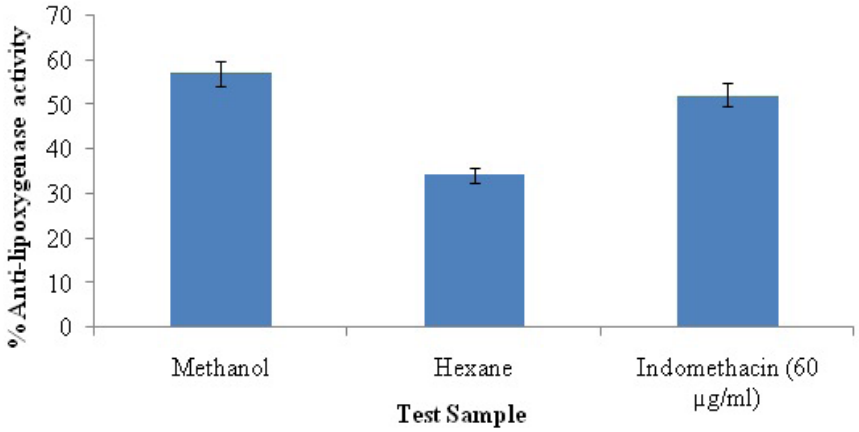

Figure 5: Anti-lipoxygenase activity of different extracts of *T. ochracea*

Table 3: Effect of different solvent extracts of *Trametes ochracea* on inhibition of xanthine oxidase and acetyl cholinesterase activities

Test Sample	Inhibitors activities (%)	
	Xanthine oxidase (IC_{50} µg/ml)	Acetyl cholinesterase
Methanol	42.36±1.24[a]	17.94±1.16[b]
Hexane	19.82±1.36[b]	7.14±1.06[c]
Galanthamine (20 µg/ml)	--	50.00±1.36[a]

Table 4: Mitotic index of methanol and hexane extract of *T. ochracea* on *Allium cepa* meristematic root cells

Samples	Mitotic index
Control	96.4±0.56
Methanol	18.1±0.08
Hexane	46.2±0.12
Lapachol	15.6±0.06

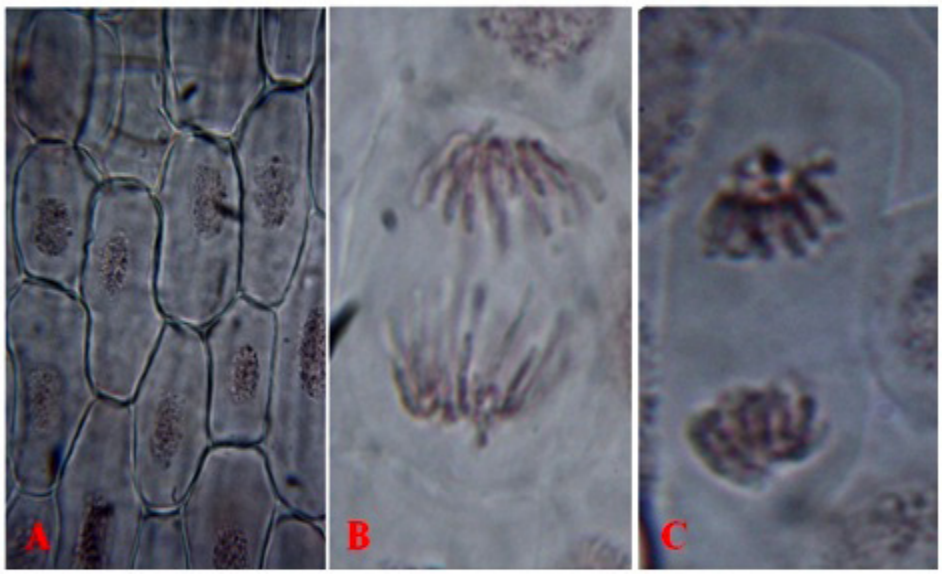

Figure 6: Normal mitotic cells A) Prophase, B) Anaphase and C) Early telophase

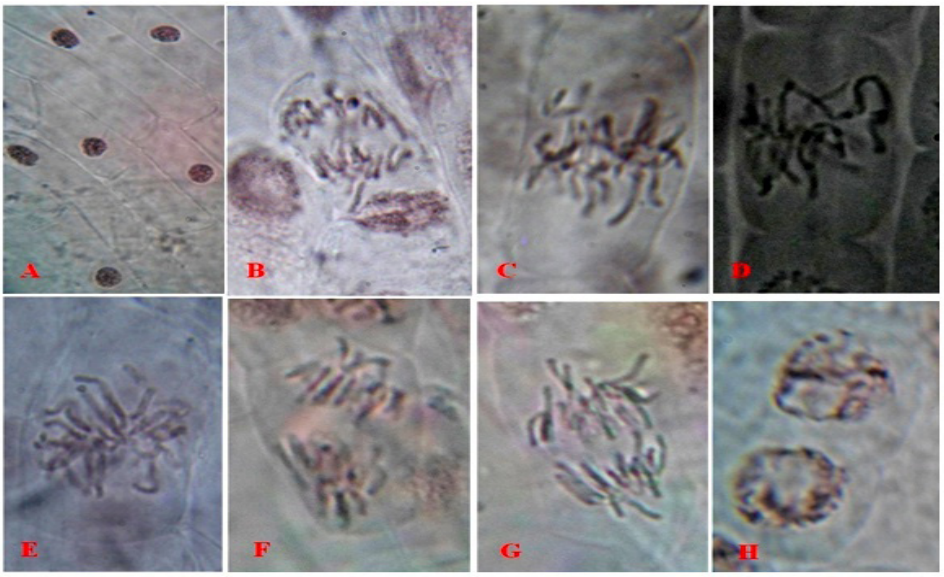

Figure 7: Abnormal mitotic cells, A) Cell shrinkage at prophase, B) Chromosomal bridges at anaphase, C) Lagging chromosome at metaphase, D) Abnormal chromosomal distribution at metaphase, E) Lagging chromosome at metaphase, F) Abnormal chromosomal distribution at anaphase, G) Chromosomal bridge H) Enlarged nucleus.

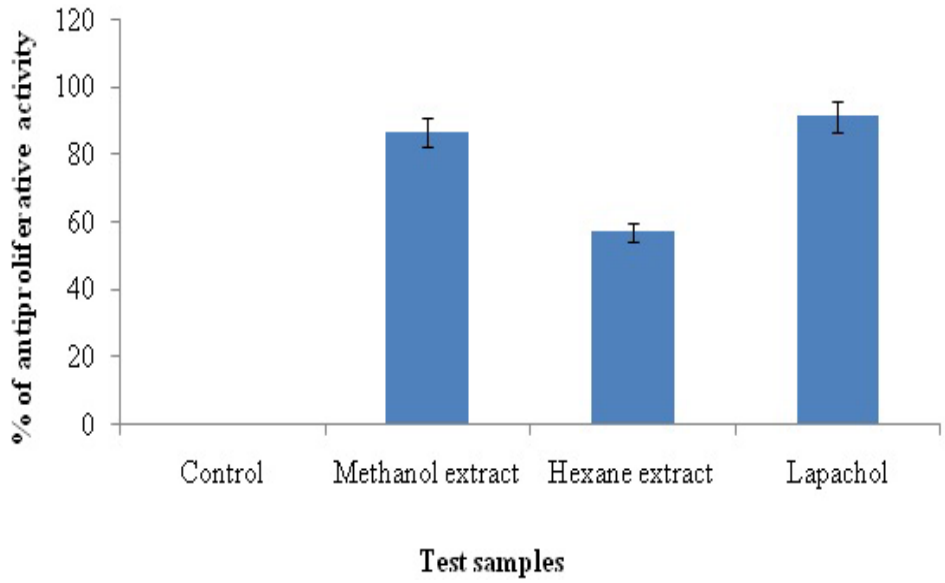

Figure 8: Antiproliferative activity of different extracts of *T. ochracea*

positive control.

The methanol (17.94 ± 1.14) and hexane (7.14 ± 1.06) extracts exhibited the best AChE inhibitory activity, but these values are lowest to galanthamine inhibitory activity (50%) at 0.2 µg/ml (Table 3).

Denaturation of proteins is a well documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone etc) have shown dose dependent ability to thermally induced protein denaturation.[26] Similar results were observed from many reports from plant extract.[27] The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutraphils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release cause further tissue inflammation and damage.[40] The precise mechanism of this membrane stabilization is yet to be elucidated; it is possible that the *T. ochracea* extracts produced this effect surface area/volume ratio of the cells, which could be brought about by an expansion of

membrane or the shrinkage of cells and an interaction with membrane proteins.[29]

Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a source of proteinase which carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes proteinase play important role in the development of tissue damage during in inflammatory reactions and significant level of protection was provided by proteinase inhibitors.[41] Recent studies have shown that many flavonoids and related polyphenols contributed significantly to the anti-inflammatory activities of many plants.[42,43] Hence, the presence of bioactive compounds in the methanol and ethanol extracts of *T.ochracea* may contribute to its, antimicrobial, antioxidant and anti-inflammatory activity.

Antimitotic activity

The methanol extract was found to best to induce antimitotic activity by reducing cell division of actively growing onion root cells at various levels of cell cycle (abnormal mitotic cells, cell shrinkage at metaphase, chromosomal bridges at anaphase, lagging chromosome at metaphase, abnormal chromosomal distribution at metaphase, lagging chromosome at metaphase, abnormal chromosomal distribution at anaphase, chromosomal bridges and enlarged nucleus) after 48 h of treatment (Figure 6). These abnormalities were observed with normal cell division stages viz., propase, anaphase, early telophase, mitotic cells (Figure 7). The activity was compared with standard anticancer agent lapachol treated cells.

We have calculated the mitotic index of the each treatment along with untreated control. The maximum mitotic index was noticed in methanol treated onion cells (18.1) followed by hexane (46.2), whereas the untreated control is possessing 96.4 mg/ml. (Table 4). Similar results were observed with different fungal species and also plant extracts.[44,45,37] The assessment of antimitotic activity using *A.cepa* root meristematic cells has been used extensively in the screening of drugs with antimitotic activity. The division in these cells is similar to normal human cells and cancer cell division. Hence, these meristematic cells can be used for screening of drugs with potential human anticancer activity. Anti-tumor drugs that interact with microtubules and tubulin are known to block mitosis and induce cell death by apoptosis.[46]

Due to the presence of different phytochemicals in methanol extract at higher concentration, it may be a single compound or in combination, the extract has showed potential antimitotic activity by inducing structural changes to chromosomes.

The methanol and hexane extracts of *T.ochracea* were evaluated for antiproliferation activity against yeast to induce inhibition of their growth. The maximum growth inhibition of yeast was observed from methanol extract (86.55). The methanol extract leads to death of yeast by inducing toxicity and death of the cells was noticed as debris or necrosis of the cells after 24 h of treatment. The hexane treated yeast cells showed less antipriliferaive acitivity (57.01) compared with methanol extract and standard anticancer agent (91.2). (Figure 8). Our results are confirmatored,[33,44,45] The yeast cell death was characterized by the number of morphological changes such as cell shrinkage, membrane blebbing, chromatic condensation, cell necrosis and formation of apoptotic bodies.[47] Yeast was selected for study of *in vitro* antiproliferative and cytotoxic assays as model system. Using chemogenomic assays in yeast,[48] showed for structurally related imidalo-pyridines and –pyrimidines a differential involvement of mitochondrial dysfunction and DNA damage in their toxicity and confirmed these results in cultured human cells. Yeast represents an inexpensive and simple alternative system to mammalian culture cells for the analysis of drug targets and for the screening of compounds in a heterologous, yet cellular, eukaryotic environment. Use yeast (*Saccharomyces cerevisiae*), the nematode *Caenorhabditis elegans*, or the fruit fly *Drosophila melanogaster*, because they share similar signaling and growth regulatory pathways with humans.[49] The advantage, particularly of yeast, is that the complete genome comprises only 6250 defined genes, and most importantly, many genes that are altered in human tumors have homologs in this model organism. The model organisms are thought to provide a valuable resource to achieve a greater understanding about human cancer and hopefully give insights into new approaches for therapy.

CONCLUSION

The methanol extract of wood rotting fungi, Trametes ochracea having important phytochemicals and exhibited potent antioxidant, anti-inflammatory, cyotoxicity activities. The activities may be in combination of all active phytochemicals or single compound.

CONFLICT OF INTEREST

We declared that we have no any conflict of interest

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