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Allium cepa L., a potential hepatoprotective and antituberculosis agent

Monika Jadhav & Pratima Tatke*

C. U. Shah College of Pharmacy, S.N.D.T. Women's University, Santacruz (W), Mumbai, Maharashtra, India

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The pharmacological effects of *Allium cepa* L., commonly known as onion, are attributed to its phenolic and flavonoid compounds. In this study, we investigated the pharmacological properties of three varieties of onion (red onion, white onion, and desiccated onion) by evaluating their antioxidant, antimicrobial, antitubercular and hepatoprotective activities. The Soxhlet extraction technique with four solvents was employed to obtain a total of 28 extracts from the onion bulb, skin, and dried powder. The extracts were subjected to various analyses, including yield determination, phytochemical testing, measurement of total phenolic contents (TPC) and total flavonoid contents (TFC), as well as *in vitro* antioxidant assays such as DPPH radical scavenging, hydroxyl radical scavenging, and superoxide radical scavenging methods. Additionally, *in vitro* antimicrobial activity of the extracts was assessed against two Gram-positive bacteria, two Gram-negative bacteria, and two fungi. The *in vitro* antitubercular activity was evaluated using the Microplate Alamar Blue Assay (MABA). Furthermore, we investigated the *in vivo* hepatoprotective activity of the bioactive extracts by using paracetamol-induced and isoniazid-rifampicin-induced hepatotoxicity models. Our results revealed that the red onion skin extracts exhibited higher levels of TPC and TFC compared to other extracts. Additionally, the red onion skin extracts displayed promising antioxidant and antimicrobial activity, while the red onion dry extracts showed significant antimicrobial and antitubercular activity which was statistically tested at 5% significant level. Notably, the hydro-alcohol extract of red onion skin and the ethyl acetate extract of dry red onion demonstrated notable antitubercular and hepatoprotective properties at $P<0.05$. These findings indicate the potential of these extracts for further comprehensive pharmacological evaluation.

Keywords: Antimicrobial,Antioxidant, *In vitro* antitubercular activity, *In vivo* hepatoprotective activity, Liver, Microplate alamar blue assay (MABA), Red onion skin extracts, Tuberculosis

Tuberculosis (TB) has become a life threatening disease from few years back and not faded away till today, though various efforts had been made globally. Globally, there are 10.6 million incident TB patients, 1.13 million deaths, 4,10,000 new cases of multi-drug resistant TB (MDR-TB) in the year 2022[1]. India is one of the high burden countries for TB with anestimated 24.2 lakh cases of TB and63,801 MDR-TB patients in 2022[2]. Directly Observed Treatment Short course strategy (DOTS) is the important strategy employed for the control of TB under the RNTCP in India. Isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), ethambutol (ETH) are used in DOTS as the main drugs with continuous administration of them at regular intervals for 6 to 9 months or even more. On the other hand, these drugs have the intense side effect of hepatotoxicity that may occur after few weeks of consumption of medications and lasts even after end of DOTS therapy. The problem of hepatotoxicity due to antitubercular drug has been studied in various cohorts at various locations globally and its incidence has been reported to be between 2% and 28%[3]. Owing to safety and efficacy of herbal medicine and its ancient background, herbal medicines are preferred for treatment of all diseases. There are review articles on the antitubercular activity of medicinal plants which reveals the potential of medicinal plants against TB[4,5].

Antitubercular potential of *Allium cepa* L. is also known since ages and frequently reported[6]. *Allium cepa* L., commonly known as onion, belongs to family *Amaryllidaceae* (formerly *Liliaceae*) and is the oldest plant used for appetizing, dietary preparations as well as for medicinal purposes. Due to presence of adequate amount of phenolics and flavonoids, onion possess antioxidant[7,8], antimicrobial[9,10], hepato-protective[11], anti-obesity[12], anticarcinogenic potentials[13,14] and enhances testosterone in males[15]. Additionally onion showed various pharmacological activities like anticholesteric, antidepressant, antidiabetic, anti-inflammatory, antiasthmatic, analgesic, hypo-lipidemic,antihypertensive, immunoprotective

*Correspondence:
E-Mail: drpratimatatke@gmail.com

and antiosteoporosis[16-18]. Multiple studies have provided evidence indicating an elevated risk of TB in individuals with chronic airway diseases (CADs) like chronic obstructive pulmonary disease (COPD)[19,20].

Clinical evidence

Research conducted by García-García *et al.*[21] has revealed that the regular intake of concentrated garlic and onion extracts can prove to be advantageous for elderly resident volunteers who suffer from infectious respiratory diseases. An alternative approach of inhalation of volatile chemicals from onion and garlic was carried out by Tan *et al.*[22] for isolated patients with mild onset infected flu. This approach was based on the biomedical effects of onion and garlic, which have been reviewed and found to have potential benefits for managing symptoms of cough, headache, and sputum production caused by virus-infected flu or similar infections. Vahid & Rahmani[23] proposed a clinical study showing that an anti-inflammatory diet can be effective in preventing or treating viral respiratory diseases. In accordance with the aforementioned experimental and clinical findings, it was hypothesized that the utilization of onion in the form of an herbal formulation derived from its extracts could potentially alleviate cough and sputum production in patients afflicted with tuberculosis. Furthermore, it is conceivable that this approach could be employed as an adjuvant in the treatment of TB, thereby contributing to the mitigation of hepatotoxicity that may arise from prolonged administration of TB medication.

In line with this postulate, here, we studied the *in vivo* hepatoprotective and *in vitro* antitubercular activity of onion using various categories of onion and solvents along with the estimation of its total phenolic, flavonoid contents and *in vitro* antioxidant, antimicrobial and antitubercular potential. *In vivo* hepatoprotective activity of selected extract was performed using paracetamol induced hepatotoxicity model of 7 days and isoniazid-rifampicin induced hepatotoxicity model of 30 days and evaluated for liver diagnostic enzymes like bilirubin, AST, ALT and alkaline phosphatase.

Material and Methods

Chemicals and reagents

Ethanol, methanol, ethyl acetate and ascorbic acid were procured from SD Fine chemicals limited. Chloramphenicol disc (10 µg/disc), nutrient agar, MGYP agar, potato dextrose agar, gallic acid, catechin, ellagic acid and quercetin were procured from himedia laboratories. Folin-Ciocalteu reagent, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), nitro blue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH), phenazine methosulphate (PMS) were procured from SRL chemicals. Liver enzymes diagnostic kits for Bilirubin, AST, ALT and alkaline phosphatase were procured from Erba chemicals. Remaining all chemicals used was of analytical grade. All readings were taken in triplicate (for *in vitro*)/6 times (for *in vivo*) and average results are presented. ANOVA test was applied within and between the groups for statistical comparison.

Collection and pre-treatment of *Allium cepa*

The study was performed on three types of *Allium cepa* that are red onion, white onion and desiccated onion. The samples of red and white onionwere collected in the month of April-May from local distributor of onion and desiccated onion was procured from local market. Red and white onion were categorised into skin, bulb and dry powder. In first category, onion bulb was taken for extraction by chopping inner bulb into small pieces. In second category outer skin of onion was separated physically and taken for extraction. In third category, bulb without skin was cut horizontally, dried under sun and grinded to get fine brown coloured powder and employed for extraction. Desiccated onion was taken as it is for extraction. Thus, three categories of each, red and white onion, and desiccated onion, made total 7 samples for the study.

Extracts preparation

Four solvents, hydroalcohol (50:50), ethanol, methanol and ethyl acetate were selected according to the nature of phytoconstituents present in onion. Extraction of 7 samples of onion (as mentioned above), using 4 solvents each was carried out using soxhlet extraction technique, and total 28 extracts were prepared by evaporating solvent in evaporating dish on water bath. The extraction conditions like volume of solvent and extraction time were optimised for each extract and final protocol of extraction was designed.

Preliminary phytochemical testing

The presence of various phytochemicals in the extracts was detected by qualitative preliminary tests as listed in the Table 1.

Table 1 — Qualitative preliminary phytochemical tests

Constituents	Tests
Alkaloids	Dragendroff's Test, Hager's Test, Mayer's Test, Wagner's test
Steroids	Salkowski reaction, Liebermann-Burchard reaction
Carbohydrates	Molisch's Test, Benedict's test, Fehling's test,
Proteins and Amino Acids	Biuret test, Million's test, Ninhydrin test
Tannins & Phenolics	5% Ferric chloride solution, 10% Lead acetate solution, 10% Potassium dichromate solution
Flavonoids	Shinoda test, alkaline reagent test
Glycosides	Legal's test, Borntrager's test, sodium picrate test, Foam test, Keller-Killani test, fluorescence test.
Gums	Acid test
Mucilage	Ruthenium red test
Fats & Oils	Oil Stain test, Saponification test

Preparation of working solution

About 10 mg extract was weighed and transferred in 10 mL volumetric flask containing 5 mL solvent. It was sonicated for 5 min and volume was made up to 10 mL with respective solvent and 1 mg/mL stock solution was obtained.

Determination of total phenolic content (TPC)

Gallic acid was used as standard for determining TPC by Folin–Ciocalteu method[24]. 0.5 mL Folin-Ciocalteu reagent was added to 1 mL of extract solution (1 mg/mL) followed by addition of 5 mL distilled water. It was incubated at RT for 10 min. Afterwards, 1.5 mL anhydrous sodium carbohydrate solution (10% w/v) was added and made final volume upto 10 mL. The mixture was allowed to stand at RT for 2 h with intermittent shaking. The absorbance of dark blue coloured solution was measured at 725 nm and results were expressed as gallic acid equivalents mg/g.

Determination of total flavonoid content (TFC)

Catechin was used as standard for determining TFC by the aluminium chloride colorimetric method[25]. About 1 mL extract solution (1 mg/mL) was taken in 10 mL volumetric flask and 4 mL distilled water was added. Afterwards, 0.3 mL of 5% $NaNO_2$ solution was added and incubated at RT for 6 min. Then, 0.3 mL 10% $AlCl_3$ solution was added and incubated at RT for 6 min. Finally, 2 mL 1M NaOH was added and immediately water was added up to 10 mL. The mixture was allowed to stand at RT for 15 min. The absorbance of pink coloured solution was measured at 510 nm and results were expressed as catechin equivalents mg/g.

Determination of *in vitro* antioxidant activity

Following three antioxidant methods were performed for determination of antioxidant activities of 28 extracts.

DPPH scavenging method

Ascorbic acid was used as standard for determining antioxidant activity by DPPH scavenging method[26]. About 3.94 mg DPPH was dissolved in 100 mL methanol to get 1 mM DPPH solution. Series of dilutions were made for every extract and standard. One mL extract solution of series dilution was taken and 3 mL DPPH solution was added, mixed and allowed to stand at RT for 30 min. The absorbance of violet coloured solution was measured at 517 nm and percentage inhibition (% In) was calculated using following formula.

$$\% \text{ In} = \frac{A_0 - A_1}{A_0} \text{ X } 100$$

where, A_0 is absorbance of blank and A_1 is absorbance of sample. Calibration curve of percentage inhibition versus concentration was plotted and 50% inhibitory concentration (IC_{50}) was calculated.

Hydroxyl radical scavenging method

Quercetin was used as standard for determining antioxidant activity by hydroxyl radical scavenging method[27]. Series of dilutions were prepared for each extract and standard. About 1 mL extract solution of series dilution was taken and in each dilution, 1 mL $FeSo_4$ solution (1.5 mM), and 0.7 mL H_2O_2 (6 mM) were added and mixed. After 2 min, 0.3 mL sodium salicylate (20 mM) was added to initiate the reaction. The mixture was allowed to stand at RT for 30 min and absorbance of orange coloured solution was measured at 562 nm. Percentage inhibition was calculated using following formula.

$$\% \text{ In} = \frac{1 - (A_1 - A_2)}{A_0} \text{ X } 100$$

where, A_0 is absorbance of blank, A_1 is absorbance of sample with sodium salicylate and A_2 is absorbance of sample without adding sodium salicylate. Calibration curve of percentage inhibition *vs*. concentration was plotted and IC_{50} was calculated.

Superoxide radical scavenging method

Quercetin was used as standard for determining antioxidant activity by superoxide radical scavenging method[28]. Series of dilutions were prepared for each extract and standard. 1 mL extract solution of series dilution was taken and in each dilution, 1 mL NBT solution (150 µM) and 1 mL NADH solution (468 µM) were added and mixed. 0.1 mL PMS (60 µM) was added to initiate the reaction. The mixture was allowed to stand at RT for 5 min and

absorbance of orange coloured solution was measured at 560 nm. Percentage inhibition was calculated using following formula.

$$\% \text{ In} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 is absorbance of blank and A_1 is absorbance of sample. Calibration curve of percentage inhibition *vs*. concentration was plotted and IC_{50} was calculated.

Determination of *in vitro* antimicrobial activity

Depending upon antioxidant study of all extracts, the extracts having better antioxidant potential were selected for antimicrobial study, which was conducted against two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*), two Gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and two fungi (*Aspergillus niger* and *Candida albicans*). Antimicrobial study was performed by disc diffusion method, disc size 6 mm, according to standard protocol[29]. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using sliding calipers or a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background and illuminated with reflected light.

Determination of antitubercular activity by Microplate alamar blue assay (MABA)

All the twenty eight extracts and three phytomarkers that are, quercetin (Q), gallic acid (GA) and ellagic acid (EA) were screened of antitubercular activity by MABA[30].

Briefly, the inoculum was prepared from fresh LJ medium re-suspended in 7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase), adjusted to a OD590 1.0, and diluted 1:20; 100 µL was used as inoculum. Each extract stock solution was thawed and diluted in 7H9-S at four-fold the final highest concentration tested. Serial two-fold dilutions of each extract were prepared directly in a sterile 96-well microtiter plate using 100 µL 7H9-S. Plates for a growth control containing no antibiotic and the standards like Isoniazid (INH), Rifampicin (RIF) and Ethambutol (ETH) were also prepared. Sterile water was added to all perimeter wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags and incubated at 37°C in normal atmosphere. After 7 days incubation, 30 µL of alamar blue solution was added to each well, and the plate was re-incubated overnight. A change in colour from blue (oxidised state) to pink (reduced) indicated the growth of bacteria, and the minimum inhibitory concentration (MIC) was defined as the lowest concentration of drug that prevented this change in colour.

***In vivo* hepatoprotective activity**

Animals

Sixty Wistar rats (thirty rats for each model) weighed between 150 and 200 g were procured for both models. The experimental protocol was assessed and approved (Approval no. CUSCP/IAEC/17/2018) by the IAEC (Institutional Animal Ethics Committee) of C. U. Shah College of Pharmacy, SNDT Women's University, Mumbai and followed the guidelines indicated by CPCSEA. Before the trial, the rats were kept in animal house for a week for acclimatization to living at 24 ± 1 °C. Standard pellet feed and purified water were made available freely to the animals. Rats were randomized into groups and housed in a group of three in sanitized polypropylene cages containing sterile paddy husk as bedding.

Isoniazid-Rifampicin (INH-RIF) induced hepatotoxicity

Hepatotoxicity was induced in Wistar rats by INH-RIF (100 mg/kg each p.o. orally for 30 days). The bioactive extracts of AC i.e. Hydro-alcohol extract of red onion skin (ROSkHA) and ethyl acetate extract of dry red onion (RODEA) at 200 mg/kg dose levels and silymarin (100 mg/kg b.w. p.o.) as standard drug were administered once daily orally for 30 days. Each group was containing six rats and the groups were divided as follows: Gr. I: Normal control - administered vehicle (distilled water); Gr. II: Negative control - INH-RIF (100 mg/kg each p.o.); Gr. III: Positive control - INH-RIF (100 mg/kg each p.o.) + silymarin (100 mg/kg); Gr. Iv: INH-RIF (100 mg/kg each p.o.) + ROSkHA (200 mg/kg); and Gr. V: INH-RIF (100 mg/kg each p.o.) + RODEA (200 mg/kg). On the last day, blood was withdrawn by retro orbital plexus for the estimation of liver diagnostic enzymes like bilirubin, AST, ALT and alkaline phosphatase.

Paracetamol induced hepatotoxicity

Hepatotoxicity was induced in Wistar rats by paracetamol (2 g/kg body wt. p.o.). ROSkHA and RODEA at dose level 200 mg/kg and silymarin (100 mg/kg body wt. p.o.) as standard drug was administered once daily orally for 7 days. Each group

was containing six rats and the groups were divided as follows: Gr. I: Normal control - administered vehicle (distilled water); Gr. II: Negative control - paracetamol (2 g/kg body wt. p.o.); Gr. III: Positive control - paracetamol (2 g/kg body wt. p.o.) + silymarin (100 mg/kg); Gr. IV: paracetamol (2 g/kg body wt. p.o.) + ROSkHA (200 mg/kg); and Gr. V: paracetamol (2 g/kg body wt. p.o.) + RODEA (200 mg/kg). On the 5th day paracetamol (2 g/kg) was administered orally to all groups except normal control group and on 7th day blood was withdrawn by retro orbital plexus for the estimation of liver diagnostic enzymes like bilirubin, AST, ALT and alkaline phosphatase.

Measurement of *in vivo* antioxidant activity

Tissue preparation

Animals were sacrificed and whole liver was isolated, washed and immediately weighed. A 10% liver homogenate was prepared separately with 1.15% KCl using homogenizer. The liver homogenate was centrifuged at 5,000 rpm for 10 min and the pellet was discarded. The supernatant was again centrifuged at 5,000 rpm for 1 h. The supernatant obtained was used for the estimation of non-enzymatic antioxidants (lipid peroxidation) and enzymatic antioxidants (superoxide and catalase).

Lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) in the liver homogenate were estimated using a standard protocol given by Ohkawa *et al.*[31]. Briefly, the liver homogenate was incubated with 8.1% SDS, 20% acetic acid (pH adjusted to 3.5 with NaOH) and 0.8% aqueous TBA at 95°C for 60 min; the mixture was cooled, added 1 mL distilled water and mixture of n-butanol: pyridine (15:1 v/v), shaken vigorously, centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer was measured at 532 nm against appropriate blank. Lipid peroxidation was determined by using formula: % inhibition = (100 – absorbance of sample)/absorbance of control × 100.

Superoxide dismutase (SOD)assay

It was carried out as per the standard protocol designed by Kakkar *et al.*[32]. Briefly, liver homogenate (0.05 mL) was taken, and 1.5 mL of 0.052 M sodium pyrophosphate buffer pH 8.3, 0.2 mL of 186 µm PMS, 0.5 mL of 300 µm NBT, and 0.3 mL of 780 µm NADH were added. The mixture was incubated at 30ºC for 1 min. The reaction was initiated by adding 1 mL of glacial acetic acid. The reaction mixture was shaken vigorously with addition of 4 mL n-butanol. It was then allowed to stand for 10 min, centrifuged and the absorbance of butanol layer was taken at 560 nm. Percent inhibition of NBT reduction by SOD was calculated according to formula given in *in vitro* antioxidant method (superoxide radical scavenging method).

Catalase (CAT) activity

As per the standard method given by Aebi[33], catalase activity was determined spectrophotometrically. To 50 µL liver homogenate, 2 mL of phosphate buffer pH 7 and 1 mL of 30 mM H_2O_2 was added, and the rate of degradation of H_2O_2 was followed at 240 nm/min. Catalase content regarding µ/mg of protein was estimated from the rate of decomposition of H_2O_2 using the formula: $k = 2.303/T \times \log (A1/A2)$, where, k is rate of reaction, T is time interval in minutes, A1 is absorbance at time zero and A2 is absorbance at 60 sec interval.

Results

Present research work was aimed to determine the total phenolic contents, total flavonoid contents, *in vitro* antioxidant, *in vitro* antimicrobial, *in vitro* antitubercular activities of 28 extracts of *Allium cepa*, *in vivo* hepatoprotective activity of two bioactive extracts.

Extraction of 7 samples (red onion bulb, red onion skin, red onion dry, white onion bulb, white onion skin, white onion dry and desiccated onion) by four solvents (hydroalcohol (50:50), ethanol, methanol and ethyl acetate) was carried out using soxhlet extraction technique. Total 28 extracts were obtained having sticky consistency and dark brown/brown/yellow colour. The solubility of each extract was determined and percentage yield (Fig. 1) was calculated. Each

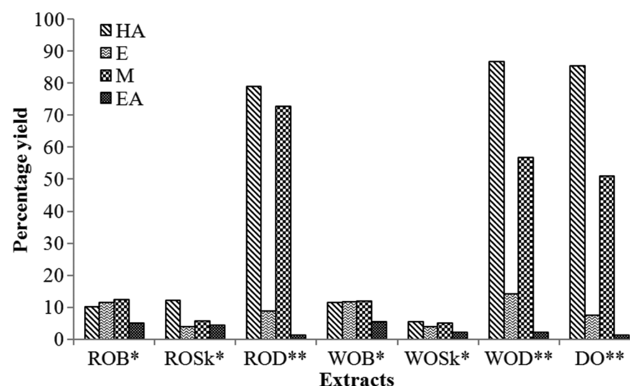

Fig. 1 — Percentage yield values of extracts (n=3, average SD=0.45) values are not *significantly different, **significantly different

extract was completely soluble in dimethyl sulfoxide and respective solubility in water, ethanol and methanol was determined (Table 2). RODHA, RODM, WODHA, WODM, DOHA and DOM showed better percentage yield than other extracts. Mostly, hydro-alcohol and methanol extracts of all samples had more percentage yield than ethanol and ethyl acetate extracts. All the percentage yield values within the group were not significantly different at 5% significance level (P<0.05); while all the mean values between the group were significantly different at 5% significance level (P<0.05).

Qualitative and quantitative phytochemical analysis

Preliminary qualitative phytochemical testing was performed for determination of various phytoconstituents present in all extracts (Table 3). All extracts showed presence of alkaloid, carbohydrates, tannins, phenols and flavonoids. Few extracts also contained steroids, proteins, glycosides and mucilage.

Quantitative determination of the phytochemicals is essential for obtaining the exact amount of phytoconstituents present in the plant extracts, which imparts pharmacological activities to them. After the qualitative phytochemical testing, estimation of total phenolic and flavonoid contents were performed. Gallic acid and catechin were used as standard for determination of TPC and TFC, respectively. A series of dilutions were prepared and a calibration curve of absorbance versus concentration was plotted. The regression equations obtained were $y = 0.1114x + 0.0419$ for gallic acid and $y = 0.1201x + 0.0159$ for catechin and these equations were used for calculation of TPC and TFC of extracts (Fig. 2).

ROSkHA, ROSkE, ROSkM, ROSkEA showed TPC of 24.92, 24.6, 26.85, 22.69 gallic acid equivalents mg/g and TFC of 2.66, 2.33, 2.81, 3.05 catechin equivalents mg/g, respectively. The amounts of phenolic and flavonoid compounds obtained in present investigation are comparable to the values reported by Albishi *et al.*[34] in the study of determination of antioxidant activity of phenolic constituent of skin and bulb of pearl, red, yellow and white coloured onions. When compared statistically, all the TPC and TFC values within the group were not significantly different at 5% significance level (P<0.05); the TPC values of ROSk were significantly different at 5% significance level (P<0.05) as compared to other extracts; the TFC values of ROSk and WOSk were significantly different at 5% significance level (P<0.05) as compared to other extracts.

Followed to that, RODEA and WOSkHA showed TPC of 6.46 and 4.36 gallic acid equivalents mg/g respectively. WOSkHA, WOSkE, WOSkM, WOSkEA, WODEA and RODEA showed TFC of 0.8, 1.38, 2.08, 1.18, 1.52 and 0.85 catechin equivalents mg/g, respectively. Thus, ROSk extracts showed higher TPC and TFC as compared to other extracts. RODEA and WOSkHA showed better TPC

Table 2 — Solubility of extracts

Extract	Solubility	Extract	Solubility
ROBHA	W,M,E	WOBM	W,M,E
ROBE	W,M,E	WOBEA	M
ROBM	W,M,E	WOSkHA	W,M,E
ROBEA	W,M	WOSkE	E
ROSkHA	W,M,E	WOSkM	M,E
ROSkE	M, E	WOSkEA	M
ROSkM	M,E	WODHA	W,M,E
ROSkEA	M	WODE	M, E
RODHA	W,M,E	WODM	W,M
RODE	W,M	WODEA	M
RODM	W,M	DOHA	W,M,E
RODEA	M	DOE	M,E
WOBHA	W,M,E	DOM	M,E
WOBE	M,E	DOEA	M

[W, water; M,methanol; E, ethanol]

Table 3 — Preliminary phytochemical testing of extracts

Name	A	S	C	P	T	P	F	G	Gm	M	F&O
ROBHA	+	+	+	+	+	+	+	+	-	+	-
ROBE	+	-	+	-	+	+	+	-	-	+	-
ROBM	+	+	+	-	+	+	+	+	-	+	-
ROBEA	+	-	+	+	+	+	+	-	-	+	-
ROSkHA	+	+	+	-	+	+	+	+	-	+	-
ROSkE	+	-	+	-	+	+	+	+	-	+	-
ROSkM	+	-	+	-	+	+	+	+	-	+	-
ROSkEA	+	-	+	-	+	+	+	+	-	+	-
RODHA	+	+	+	-	+	+	+	-	-	+	-
RODE	+	+	+	-	+	+	+	-	-	+	-
RODM	+	+	+	-	+	+	+	-	-	+	-
RODEA	+	-	+	-	+	+	+	-	-	-	-
WOBHA	+	+	+	+	+	+	+	-	+	+	-
WOBE	+	+	+	-	+	+	+	-	-	+	-
WOBM	+	+	+	-	+	+	+	-	+	+	-
WOBEA	+	+	+	-	+	+	+	-	+	+	-
WOSkHA	+	-	+	-	+	+	+	-	+	+	-
WOSkE	+	-	+	-	+	+	+	-	+	+	-
WOSkM	+	-	+	-	+	+	+	-	+	+	-
WOSkEA	+	-	+	-	+	+	+	-	+	+	-
WODHA	+	+	+	+	+	+	+	+	+	+	-
WODE	+	+	+	-	+	+	+	+	+	+	-
WODM	+	+	+	-	+	+	+	+	+	+	-
WODEA	+	+	+	-	+	+	+	-	+	-	-
DOHA	+	-	+	-	+	+	+	+	+	+	-
DOE	+	-	+	-	+	+	+	-	+	+	-
DOM	+	-	+	-	+	+	+	-	+	+	-
DOEA	+	-	+	-	+	+	+	-	-	-	-

[A, Alkaloids; S, Steroids; C, Carbohydrates; P, Proteins; T, Tannins; P, Phenolics; F, Flavonoids; G, Glycosides; Gm, Gums; M, Mucilage; F&O, Fats & Oils]

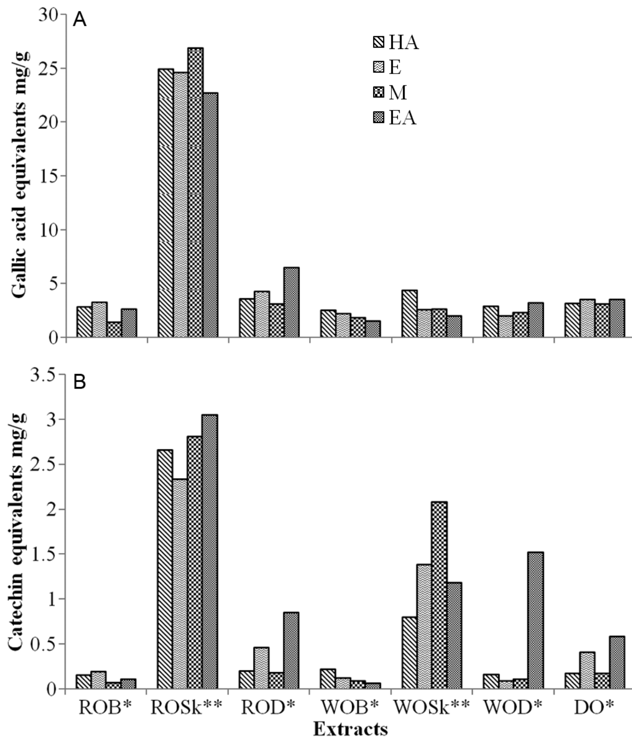

Fig. 2 — (A) Total phenolic content; and (B) Total flavonoid content of extracts (n=3, average SD TPC=0.14, average SD TFC=0.022) *values are not significantly different, ** values are significantly different

after ROSk extracts. RODEA, WODEA and WOSk extracts showed less TFC than ROSk but higher than other extracts.

***In vitro* antioxidant activity**

Evaluation of *in vitro* antioxidant activity of all extracts was done by three methods and 50% inhibitory concentration (IC_{50}) was calculated. In DPPH scavenging method (Fig. 3A), ascorbic acid (standard) showed IC_{50}11.76 µg/mL and that of ROSkHA, ROSkE, ROSkM, ROSkEA was 53.23, 48.77, 17.67, 35.85 µg/mL, respectively. In hydroxyl radical scavenging method (Fig. 3B), quercetin (standard) showed IC_{50} 118.4 µg/mL and that of ROSkHA, ROSkE, ROSkM, ROSkEA was 165.7, 493.5, 139.6 and 442.8 µg/mL, respectively. In superoxide radical scavenging method (Fig. 3C), quercetin showed IC_{50} of 223.6 µg/mL and ROSkHA, ROSkE, ROSkM, ROSkEA showed IC_{50} of 2485.6, 1845.4, 1538.6, 7426.9 µg/mL, respectively. Remaining all extracts showed less antioxidant activity than ROSk extracts. All the calculated values of antioxidant activity within the group were not significantly different at 5% significance level ($P<0.05$) and the antioxidant values of ROSk were significantly different at 5% significance level ($P<0.05$) as compared to other extracts.

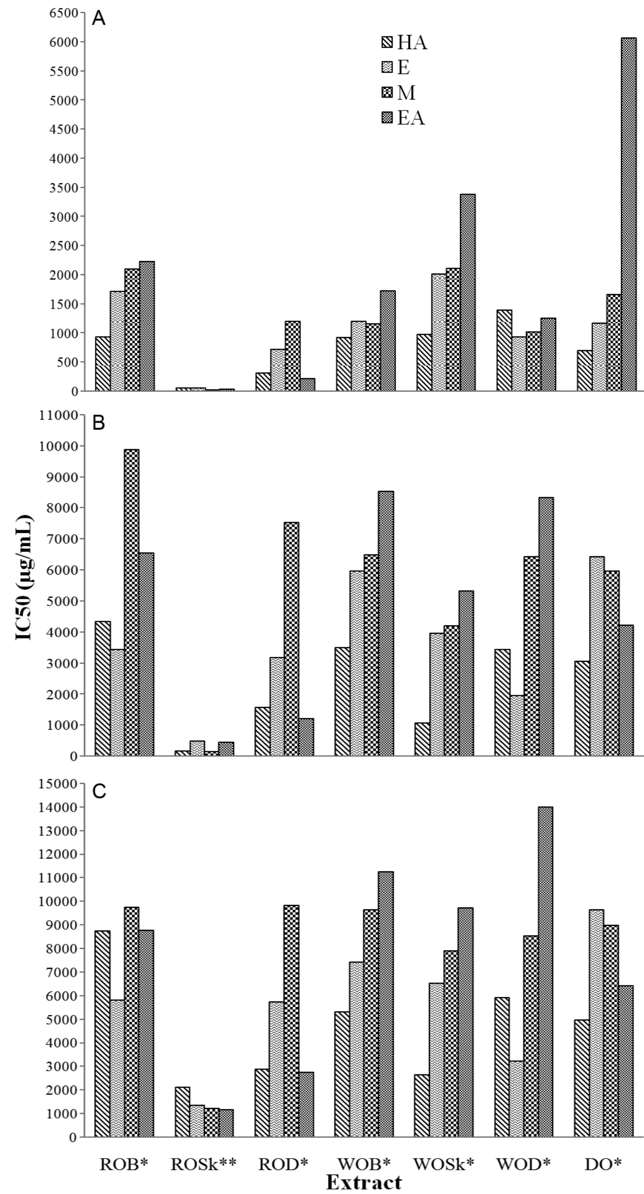

Fig. 3 — IC_{50} values of extracts for *in vitro* antioxidant activity (n=3). (A) DPPH scavenging (n=3, average SD=28.9); (B) Hydroxyl radical scavenging (n=3, average SD=81.1); and (C) Superoxide radical scavenging (n=3, average SD=149.8) [*values are not significantly different, ** values are significantly different]

Among 28 extracts of various samples of onion, ROSkM had better antioxidant activity in all methods, followed by ROSkHA, ROSkE and then ROSkEA. The extracts of ROD were showing good antioxidant activity after ROSk extracts, while extracts of ROB had less antioxidant activity than extracts of ROSk and ROD. Among the extracts of white onion, extracts of WOD were showing more antioxidant

activity than extracts of WOB and WOSk, but less antioxidant activity than extracts of red onion. Desiccated onion extracts were showing more antioxidant activity than extracts of white onion, but less antioxidant activity than extracts of red onion. With respect to the solvents used, methanol and hydroalcohol extracts were having good antioxidant activity than ethanol and ethyl acetate extracts.

***In vitro* antimicrobial activity**

Antioxidant potential of ROSk, ROD and WOB extracts was found to be better than other extracts and thus, these 12 extracts were selected for antimicrobial activity. Disc diffusion method was used for determination of antimicrobial susceptibility of extracts and zone of inhibition was measured as plotted in Fig. 4. All the calculated values of antimicrobial activity within the group were not significantly different at 5% significance level ($P<0.05$) and the antimicrobial values of standard group were significantly different at 5% significance level ($P<0.05$) as compared to all other extracts.

All the extracts showed antimicrobial activity against *E. coli*, of which ROD extracts showed better antibacterial activity followed by ROSk and WOB extracts. Similarly all extracts except WOBHA showed antibacterial activity against *B. subtilis* with more inhibition of growth by ROD extracts. The growth of *P. aeruginosa* and *S. aureus* was inhibited by ROD extracts with larger zones of inhibition, indicating its higher antibacterial potential than ROSk and WOB extracts. Antifugal activity was exhibited by few extracts only, of which ROSkE, ROSkM, RODEA, WOBHA and WOBE showed antifungal activity against *C. albicans* while that of ROSkE, ROSkM, WOBE, ROSkHA and WOBEA against *A. niger*, in decreasing order. Thus, among all extracts, ROD extracts showed better antimicrobial activity followed by ROSk and then WOB extracts.

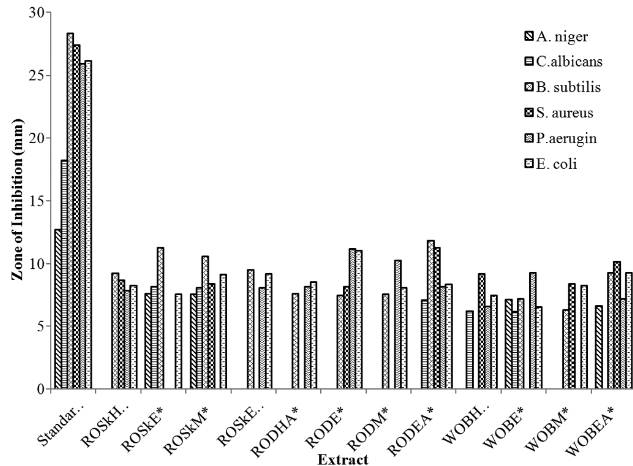

Fig. 4 — *In vitro* antimicrobial study (n=3, SD=5.08) [*values are not significantly different, ** values are significantly different]

***In vitro* antitubercular activity**

In the present investigation, evaluation of antitubercular activity was performed using MABA in which MIC of extracts against *Mtb*H37Rv was calculated. INH, RIF and ETH were used as standard for MABA, which showed MIC 0.05, 0.1 and 1.56 µM, respectively. Three phytomarkers, Q, GA, EA showed MIC 25, 25 and 12.5 µM, respectively. Among all the extracts, RODHA, RODEA, WOBE showed MIC 25 µM, while remaining all extracts were having MIC >25 µM.

***In vivo* hepatoprotective activity**

With respect to the observations from *in vitro* antioxidant, antimicrobial and antitubercular activity, hydro-alcohol extract of red onion skin and ethyl acetate extract of red onion dry were selected for *in vivo* hepatoprotective activity.

Paracetamol and INH-RIF caused significant elevation ($P<0.05$) in the levels of ALT, AST, ALP and bilirubin as compared to normal control in both models. ROSkHA and RODEA at a dose of 200 mg/kg significantly reversed ($P<0.05$) the elevation in the level of ALT, AST, ALP and bilirubin, respectively in both models as shown in Fig. 5. These effects were comparable, and not significantly different ($P>0.05$), from those elicited by the control and silymarin, which also significantly reversed ($P<0.05$) the elevation of ALT, AST, ALP and bilirubin produced by the hepatotoxicant.

Paracetamol and INH-RIF caused significant ($P<0.05$) reductions in the level of CAT, SOD, while

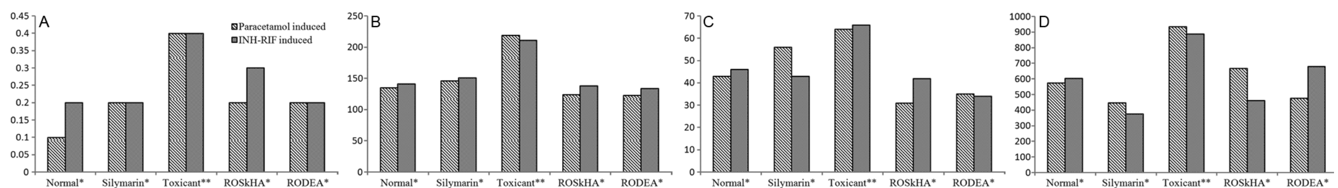

Fig. 5 — *In vivo* hepatoprotective activity (n=6); (A) Total bilirubin; (B) AST/SGOT; (C) ALT/SGPT; and (D) ALP. [*values are not significantly different, ** values are significantly different]

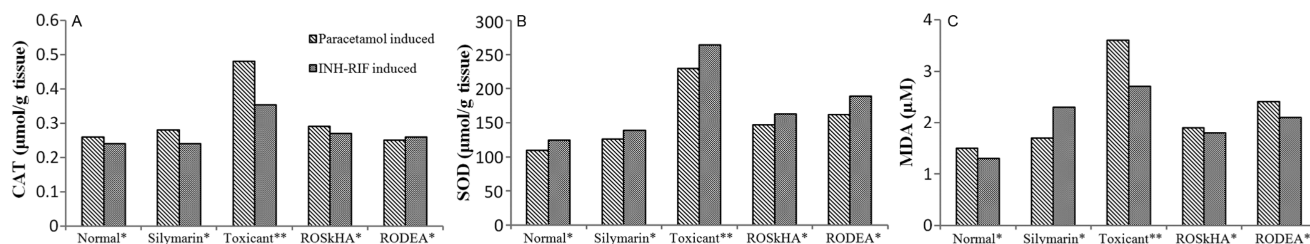

Fig. 6 — *In vivo* antioxidant activity (n=4). (A) CAT; (B) SOD; and (C) TBARS. [*values are not significantly different, ** values are significantly different]

causing a significant increase in the level of MDA as shown in Fig. 6. ROSkHA and RODEA at the dose of 200 mg/kg significantly reversed ($P<0.05$) the diminution produced by the Paracetamol and INH-RIF. Silymarin, ROSkHA and RODEA significantly increased ($P<0.05$) the level of CAT and SOD, while significantly reduced the level of MDA compared to the hepatotoxicant only group. The effects of ROSkHA and RODEA were comparable, and not significantly different ($P>0.05$) from that of the standard hepatoprotective drug, silymarin.

Discussion

TB is the main cause of morbidity in modern era and the synthetic drugs used in the treatment of TB are effective, but many of these are associated with the problem of hepatotoxicity. In contrast to this, medicinal plants can exhibit the new molecular entities having antitubercular potential without any side effects. In view of finding such potential and after completing vast literature search, *Allium cepa* was selected for this project, which possesses antimycobacterial, hepatoprotective and many pharmacological activities and widely used for development of nutraceuticals and value-added products.

ROSk extracts contained more amounts of phytoconstituents than other extracts which indicated that ROSk extracts had more bioactive substances that could show biological significance. It is studied by Hirota *et al.*[35] that the most important flavonol present in onion is quercetin and its concentration in onion goes on decreasing from outer to inner layers due to prevention of onion bulb against UV radiations and intracellular hydrogen peroxide formed in plant cells cooperating with peroxidase and ascorbic acid. Another researcher, Takahama *et al.*[36] reported that the outer layer of yellow onion gets converted to brown on maturation, whereas white onion bulbs do not have changes in the colour of their outer layer, which makes yellow onion more resistant to infection than white onion. This can be correlated with our findings in qualitative phytochemical testing that, red onion and its skin contained more amounts of tannins, phenols and flavonoids than white and desiccated onion.

Patil *et al.*[37] studied distribution of bound and free quercetin among different onion rings for which they separated the dry skin, outer rings, and inner rings and extracted with ethanol to obtain quercetin, concluding that outer layer of onion (skin) contained higher concentration of quercetin. The similar remarks were given by Sidhu *et al.* [38] who recently evaluated antioxidant potential of six different cultivars of onions from Kuwait. Thus, our results are in line with the reported outcomes, which signify the presence of exceeding amounts of phenolic and flavonoid contents in red onion skin.

Physiological and biochemical processes in human body produce free radicals. The unpaired electrons of these free radicals make them highly unstable. These unstable molecules always try to attract electron from other molecules that leads to oxidative damage of biomolecules such as lipids, proteins and DNA, resulting in various diseased conditions. Antioxidants play a crucial role in stabilizing these free radicals which prevents oxidative damage of biomolecules and protects the body from oxidative stress[26]. It has also been studied that synthetic antioxidants are associated with some side effects and hence natural antioxidants are in demand. Plants produce secondary metabolites like flavonoids which are well known source of natural antioxidants[39].

Allium cepa contains high amount of phenolics and flavonoids, but the relative study of different varieties and different parts of onion showing the respective amounts of bioactive phytoconstituents is reported by very few researchers. According to literature, comparative studies between various varieties of onion showed that red onion has more amounts of flavonoids like quercetin and hence strong antioxidant

and antitumor/anti-infection potential than white, yellow and violet onion[16,18,40]. Considering the need for further exploration for correlative study between various varieties and parts of onion, current study was planned to be performed on red, white and desiccated variety along with distinct parts like bulb, skin and dry powder of onion.

Management of infections in patients is associated with bacterial resistance to antimicrobials, which suggests that the testing of antimicrobial activity of compounds is mandatory to know their susceptibility towards various microorganisms. There are various methods available for determination of antimicrobial susceptibility, such as cup plate method, ditch plate method, disc diffusion method, antimicrobial gradient method, broth dilution method etc. The disk diffusion susceptibility method is comparatively simple, economic, practical and well standardized. The advantages of the disk diffusion method include its simplicity for performing the test as well as simplicity in interpretation of results. The disadvantages of this method are the lack of automation of the test and it cannot be used for all fastidious or slow growing bacteria. The testing of some microorganisms needs the use of specialized media, incubation conditions, and specific zone size interpretive criteria. But it can easily and routinely be used for most of gram positive, gram negative and fungi[29].

Santas *et al.*[9] concluded that onion skin extracts were having significantly stronger inhibitory effects against selected bacteria and fungi, than onion bulb extracts. Methanolic extracts of inner layers of red onion showed 12, 9 and 10 mm against *E. coli*, *P. aeruginosa* and *S. aureus*,respectively[41], and these results are nearly equivalent to the results obtained in present antimicrobial study. Srinivasan *et al.*[42] studied antimicrobial activity of aqueous onion extract in various microorganisms, and zones of inhibition shown by onion against*E. coli*, *B. subtilis*,*S. aureus*,*P. aeruginosa*,*C. albicans* and*A. niger*were 21, 21, 18, 22, 23 and 18, respectivelywhich is in agreement with results obtained in the present study.

Gupta *et al.*[6] reported first time the antitubercular activity of onion against MDR isolates, in which antimicrobial assays were performed in Lowenstein Jensen (L-J) medium and Middlebrook 7H9 broth in BacT/ALERT 3D system and aqueous extract of onion showed antitubercular activity of 37% for MDR isolate DKU-156, 79% for MDR isolate JAL-1236 and 35% for sensitive *Mtb*H37Rv. In another study, Sivakumar & Jayaraman[43] have demonstrated inhibition by aqueous and ethanolic extracts of onion at 100 µg onwards to the higher concentrations by MABA assay. All the reported studies for evaluation of antitubercular activity of onion were conducted for aqueous and ethanolic extracts of only one variety of onion. The present research work explored three varieties as well as extracts using four solvents.

Over all, the results obtained from preliminary phytochemical testing, TPC, TFC and *in vitro* antioxidant activity showed that red onion skin extracts are having promising antioxidant activity as compared to extracts of bulb and dry onion of all varieties (red, white and desiccated onion). However, the results of antimicrobial study stated that red onion dry extracts are having better antibacterial as well as antifungal potential followed by red onion skin and white onion bulb extracts. Hydroalcohol and ethyl acetate extracts of red onion dry and ethanol extract of white onion bulb showed significant antitubercular activity among all extracts. The results obtained for *in vivo* hepatoprotective activity suggests that the hydroalcohol extract of red onion skin and ethyl acetate extract of red onion dry possesses hepato-protective activity with enhancement of *in vivo* antioxidant activity as one of the possible mechanisms of action. Thus, red onion extracts, specifically red onion skin and dry extracts, can be further studied for antitubercular potential against multidrug resistant and totally drug resistant strains and could find utilisation as adjuvant for antitubercular therapy.

Conclusion

Extracts of onions, which have been prepared using different solvents, exhibited noteworthy antioxidant, antimicrobial, antitubercular and hepatoprotective properties. An assessment of these extracts has revealed that they have the capacity to impede the onset and progression of TB infection and mitigate the risk of liver toxicity. Therefore, it can be concluded that these extracts could be used in conjunction with TB treatment, which would have the added benefit of protecting liver function and lowering patient mortality rates.

Conflict of Interest

Authors declare no competing interests.

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