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Original Article

ASSESSING THE THERAPEUTIC ROLE OF *JOSHANDA*: PHYTOCHEMICAL, ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITIES

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ABSTRACT

Objective: *Joshanda,* a polyherbal Unani formulation is extensively used as a common home remedy for the treatment of a cough and cold accompanied by pharyngeal inflammation and fever. This study aimed to analyze phytochemicals, antioxidant, anti-inflammatory, and antibacterial activity.

Methods: The study investigated the presence of phyto-compounds in *joshanda* and antioxidant, antibacterial, anti-fungal, and anti-inflammatory activities by various *in vitro* standard methods using ascorbic acid, ampicillin, and aspirin respectively as standard drugs.

Results: *Joshanda* aqueous extract revealed the presence of tannins, phenols, flavonoids glycosides, terpenoids, and alkaloids and absence of sterols, saponins, xanthoprotiens, and carboxylic acid. J*oshanda* showed the highest inhibition against *B. subtilis* (% MGI 99.000±0.577) and least inhibition against *P. aeruginosa* (%MGI of 84.102±0.491). *Joshanda* extract, ascorbic acid demonstrated highest % DPPH radical scavenging of 98.379±0.313%, 98.843±0.443% and a minimum of 36.210±1.174%, 83.192±0.422%. Results showed H_2O_2 scavenging activity of 0.047±0.001 µg/ml per minute degradation of H_2O_2. FRAP value was observed in *joshanda* and ascorbic acid with a maximum of 0.945±0.024, 0.687±0.047 mmol and minimum of 0.171±0.036, 0.059±0.005 mmol respectively. J*oshanda* extract showed the highest albumin denaturation inhibition of 14.069±0.350% and the lowest of 1.880±0.194% at extract volume of 1000 µl and 100 µl respectively. The extract demonstrated the highest proteinase inhibition of 24.003±0.291 % and the lowest of 4.959±0.254% comparable to aspirin. J*oshanda* had no potent anticandidal activity up to 1 mg/ml.

Conclusion: Results clearly suggested that *joshanda* is a potent phytodrug and can also be used as a strong reactive oxygen species scavenger, might be used as anti-arthritic and strong natural antibiotic agent for effective treatment of various oxidative stressed disorders.

Keywords: *Joshanda*, Antioxidant, Antibacterial, Anti-inflammatory, Unani, Biological relevance

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INTRODUCTION

In the recent years, there has been a great switchover in the universal trend of medicine selection from synthetic to herbal medicine, which indicates "Return to Nature". Medicinal plants have been best known for millennial and are highly important all over the world as a rich source of therapeutic agents for the prevention and cure of diseases and ailments [1]. The global rise in demand for herbal medicines has led to the decline in their quality as there is a lack of adequate regulations pertaining to drugs [2]. World health organization (WHO) has highly emphasized the need to ensure quality control of medicinal plant products by the use of modern techniques and by applying suitable parameters and standards. In order to overcome inevitable shortcomings of the pharmacopeia monograph other different quality control measures must be explored. [3–6]. Out of 255 drugs (which are considered as basic and essential by the WHO, 11% is extracted from plants, and many synthetic drugs are also extracted from natural precursors.

Phytochemicals are known to possess antioxidant, antibacterial, antifungal, antidiuretic, anti-inflammatory, and radioprotective activity [7-13], and due to these properties, they are largely used and given preference for medicinal purpose. The development of drug resistance and the undesirable side effects of certain antibiotics have led to the search for new antimicrobial agents, mainly among plant kingdom, to find leads with unique chemical structures which may exert a hitherto unexploited mode of action. Obtaining potential and basic benefits from plants, always been a field of speculation for researchers and has formed the basis for the development of drugs to treat various diseases. Henceforth, screening of plants for the presence of natural products and beneficial properties presents a major avenue. The resistance acquired by microbes to the existing antibiotics demands increased efforts in the development of new antibiotics. Although various plants with antimicrobial potential have been identified, a great number still remains unidentified. High range of climatic variation from tropical to alpine leads to the richness in biological diversity. Many kinds of plants are pervasive in India and many of them have been used for antimicrobial assay [14]. There is a dire need of extensive hard studies of medicinal plants found with a special reference to their properties to fight against microbial diseases. Therefore, qualitative phytochemical screening of these phyotdrugs is a step towards 'cure by nature'. "*Joshanda*", a Persian word with a meaning "prepare by boiling". Unani medicines are usually taken as aqueous extracts containing some water-soluble organic principles and mostly inorganic ion compounds. The most frequently used formulation of joshanda consists of seven ingredients [35]. *Joshanda* has been especially used in the treatment of cold, cough, and related allergic disorders. It has been reported to possess antihistamine, antitussive, expectorant, antipyretic, and anti-inflammatory activities [36, 37]. The main composition of *joshanda* has been given in table 1. In the market, joshanda is available in the form of a dry mixture.

This article aims to assess the presence of phytochemicals in the aqueous extract of *joshanda*. Further, we have assessed the *in vitro* antioxidant, anti-inflammatory, antibacterial, and antifungal activities of the aqueous extract of *joshanda.* This is the first report (to the best of our knowledge) till date on this kind of study where the aqueous extract of *joshanda* was evaluated for various phyto-compounds and biological activities.

Table 1: The main composition of *joshanda*

S. No.	Scientific name	Common name	Part used
1.	*Althea officinalis*	*Khatmi*	seeds
2.	*Cordia latifolia*	*Sapistan*	dried fruit
3.	*Glycyrrhiza glabra*	*Mulethi*	dried rhizomes
4.	*Malvaro tundifolia*	*Khubbazi*	seeds
5.	*Onosma bracteatum*	*Gaozaban*	leaves
6.	*Viola odorata*	*Banafsha*	flowers
7.	*Zizyphus jujuba*	*Unnab*	dried fruit

MATERIALS AND METHODS

Sample preparation

10 gm of *joshanda* with all seven components in equimolar ratio were boiled in 200 ml of distilled water at 100 °C for 30 min to make a decoction of final volume 100 ml. The extract was then filtered using a muslin cloth. The filtrate was then centrifuged and the supernatant was obtained. This served as an aqueous extract of *joshanda* for the further phytochemical screening and *in vitro* biological studies.

Chemicals and reagents

All the chemicals (analytical grade) used for phytochemical screening, antioxidant, anti-inflammatory and antibacterial assay were purchased from Merck, SRL, and Himedia. DPPH (1, 1-diphenyl-2-picrylhydrazyl) and TPTZ (2, 4, 6-tris (2-pyridyl)-S-triazine) were purchased from Sigma-Aldrich.

Phytochemical screening

Various standardized qualitative chemical tests were performed for qualitative determination of different phytoconstituents present in the aqueous extract of *joshanda* by the method of Harborne with some modifications [38].

Evaluation of antibacterial potential of *joshanda*

Test microorganisms

The basic four clinical isolates of bacteria used for the study are *Staphylococcus aureus* (MTCC 902), *Escherichia coli* (MTCC 443), *and Bacillus subtilis* (MTCC 736), *Pseudomonas aeruginosa* (MTCC 2453). Their cultures were procured from NCCS, Pune, India and maintained on nutrient agar plates at 4 °C.

Broth dilution method

Antimicrobial activity of the aqueous extract of *joshanda* was tested against four bacterial strains, out of which two of them were gram-positive bacteria (*B. subtilis* and *S. aureus*) and the other two were gram-negative bacteria (*E. coli* and *P. aeruginosa*) by the method of Barbade and Datar with some modifications [15]. Cultures were prepared overnight in Luria broth (LB) media by inoculation with a single colony from agar plates and incubated for 12 h at 37 °C. These cultures were diluted with fresh LB media to approximately 10^4 colony forming units (CFU) and incubated at 37 °C for 12-14 h in the presence of *joshanda* extract analogized to the growth of the control culture where media and bacterial inoculums were only taken. The experiment was performed in triplicates (n=3). The percentage inhibition was calculated by using the following formula.

$$\text{Mean Growth Inhibition (\%)} =[(dc-dt)/dc] \times 100$$

Where dc and dt represent the absorbance of control and treated sample strains at 600 nm respectively.

Agar well diffusion method

To determine the antibacterial activity of *joshanda*, agar well diffusion method was used. The log phase bacterial cultures (secondary culture) was spread on LB agar medium plates by using a sterile spreader in order to get a uniform bacterial growth on test plates. A sterile cork borer was used to punch the wells over the agar plates. About 10-20 µl of each extract was added using sterile syringe into wells and kept at room temperature for 2h for diffusion. Ampicillin (10 mg/ml) was used as the standard antibacterial drug. The plates were then incubated at 37 °C for 18-24 h. The diameter of the inhibition zone (mm) was calculated. The results (zone of inhibition) were compared with the activity of the standard. The experiment was repeated 2 times for the confirmation

Determination of anti-inflammatory activity

Inhibition of albumin denaturation

The anti-inflammatory activity by inhibition of albumin denaturation was done using the method of Mizushima *et al.* [16] with minor changes. A wide range of diluted working solutions of the *joshanda* and its constituents plants were prepared and mixed with 1% aqueous solution of bovine serum albumin (BSA) fraction. The samples were incubated at 37 °C for 20 min and then heated at 57 °C for 20 min. 1% BSA was taken as control and Tris buffer was taken as blank. Aspirin (100 µg/ml) was taken as a standard drug. After few minutes of cooling the samples, the turbidity was studied at 660 nm. The experiment was performed in triplicates (n=3). Percent inhibition of protein denaturation was calculated by the formula.

$$\text{\% inhibition} =(\text{Abs control}-\text{Abs sample}) \times 100/\text{Abs control}$$

Proteinase inhibitory action

The experiment was executed according to the revised procedure of Oyedepo *et al.* [17]. The reaction mixture (2 ml) contained 1 ml 20 mmol Tris HCl buffer (pH 7.4), 1 ml test sample of different concentrations, and 0.06 mg trypsin. The mixture was incubated at 37 °C for 5 min followed by addition of 1 ml of 0.8% (w/v) casein. The mixture was again incubated for further 20 min. At the end, 2 ml of 70% perchloric acid was added to finish the reaction. The absorbance of the supernatant was read at 210 nm against buffer as blank, after removing the cloudy suspensions through the centrifuge. The experiment was performed in triplicates (n=3). Further, the percentage inhibition of proteinase inhibitory activity was determined by the following formula.

$$\text{\% proteinase inhibition}=(\text{Abscontrol}-\text{Absbsample}) \times 100/\text{Abscontrol}$$

Determination of antioxidant activity

Catalase (CAT) assay

Catalase activity was assayed following the method of Jambunathan *et al.* with minor modification [18]. H_2O_2-Phosphate buffer (hydrogen peroxide-phosphate buffer) (3.0 ml) was taken in an experimental cuvette, followed by the rapid addition of 40 µl of *joshanda* and mixed thoroughly. The time required for a drop in absorbance by 0.05 units was noted at 240 nm. The H_2O_2-Phosphate buffer was served as control. CAT activity in each sample was expressed in nmol/min/ml. One unit was defined as the amount of enzyme that caused the formation of 1.0 nmol of formaldehyde per minute at 25 °C. The experiment was performed in triplicates (n=3).

1, 1 Diphenyl-1-(2, 4, 6-trinitrophenyl) hydrazyl (DPPH) assay

The antioxidant activity of *joshanda* was checked on the basis of the free radical scavenging effect of the stable DPPH according to the protocol of Goveas and Abraham with minor modifications [19]. A wide range of diluted working solutions of *joshanda* was prepared in distilled water and methanol respectively. 0.1 mmol DPPH was prepared in 80% methanol and 500 µl of this solution was mixed with 500 µl of working sample solutions and standard solution separately. Ascorbic acid (1 mg/ml) in distilled water was used as the standard. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm. 0.1 mmol DPPH solution was used as the control. The range of diluted aqueous extracts was taken as blank. The experiment was performed in triplicates (n=3). The optical density was recorded and DPPH scavenging was calculated using the given formula.

DPPH scavenging Activity (%)=[(dc-dt)/dc] x 100

Where dc and dt represent absorbance of control and test sample respectively at 517 nm.

Ferric reducing antioxidant power (FRAP) assay

Antioxidant activity assay was assessed based on the FRAP assay by the method of Sudha *et al.* with minor modification [20]. FRAP reagents were freshly prepared by mixing 10 ml acetate buffer (300 mmol, pH 3.6), 1 ml TPTZ solution (10 mmol TPTZ in 40 mmol/l HCl) and 1 ml $FeCl_3$ (20 mmol) water solution. A range of diluted working solutions of the *joshanda* was prepared in distilled water. Each sample (200 µl) was added in 1.5 ml of freshly prepared FRAP reagent and mixed and after 5 min, absorbance was measured at 593 nm, using the working solution of FRAP as blank. The standard antioxidant used was ascorbic acid. The results were expressed in mmol Fe^{2+}/ml of aqueous extract. The experiment was performed in triplicates (n=3). Higher absorbance indicated higher reducing power.

Estimation of reduced glutathione (GSH)

Reduced glutathione was estimated by following the method described by Moron *et al.* [21]. Reduced glutathione, when reacted with DTNB (5, 5'-dithiobis nitrobenzoic acid), gave a yellow colored product that absorbed at 412 nm. A homogenate was prepared with 0.5 g of the plant sample along with 2.5 ml of 5% trichloroacetic acid (TCA). The precipitated protein was centrifuged at 1000 rpm for 10 min. The supernatant (0.1 ml) of the mixture was further used for the determination of GSH. The supernatant (0.1 ml) was composed of 1.0 ml with 0.2 M sodium phosphate buffer (pH 8.0). Standard GSH corresponding to concentrations ranging between 2 and 10 moles was also prepared. Two ml of freshly prepared DTNB solution was added and the intensity of the yellow color developed was measured in a spectrophotometer at 412 nm after 10 min. The values were expressed as n moles GSH/g sample. The experiment was performed in triplicates (n=3).

Determination of antifungal activity

The determination of the minimum inhibitory concentration of joshanda extract against *Candida* cells (ATCC 10261 and ATCC 90028) by broth dilution was carried by the method given by the Clinical and laboratory standards institute (CLSI). Different cultures were grown with or without test compounds in the media. Two-fold dilutions of the test compound were carried out as an initial step. *Candida* cells (10^5cells/ml) were inoculated in molten yeast extract peptone dextrose (YEPD) agar (~ 40 °C) and poured into Petri plates. Also, the antifungal activity of aqueous extract in solid media was determined by the disc diffusion method. Different concentrations of test compounds were spread on the disc placed on solid agar in 10 µl volume. The average diameter of the zone of inhibition was measured in millimetres. The experiment was performed in triplicates (n=3).

RESULTS

Phytochemical screening

The phytochemical analysis of the aqueous extract of *joshanda* revealed the presence of phytochemicals. An adequate amount of tannins, phenols, flavonoids glycosides, terpenoids, and alkaloids were found in the aqueous extract. However, screening depicted the absence of sterols, saponins, xanthoprotiens, and carboxylic acid. The results obtained are depicted in table 2.

Table 2: Phytochemical composition of aqueous extract of *joshanda*

S. No.	Phytochemical constituent	Result
1.	Terpenoids	Positive
2.	Phenols	Positive
3.	Carboxylic acid	Negative
4.	Flavonoids	Positive
5.	Glycosides	Positive
6.	Xanthoproteins	Negative
7.	Tannins	Positive
8	Sterol	Negative
9.	Saponins	Negative
10.	Alkaloids	Positive

Determination of antibacterial activity of *joshanda*

Broth dilution assay

Antibacterial assay of *joshanda* was examined against various bacterial strains by accessing the percentage inhibition in presence of the extract. A range of various concentrations of extract was tested against four different strains of bacteria. It was found that *joshanda* had strongest inhibitory activity against *B. subtilis* with %MGI of 99.000±0.577 whereas; it showed the least inhibition against *P. aeruginosa* with % MGI of 84.102±0.491. The results were quite comparable to ampicillin which showed almost complete inhibition against all the bacterial strain. The extent of inhibition increased with length of incubation and increase in concentration. Hence, the results showed that *joshanda* exhibit bactericidal property *in vitro* i.e. the growth of microorganisms was inhibited in its presence in time and concentration-dependent manner (fig. 1).

Well diffusion assay

The aqueous extract of *joshanda* was also tested for antibacterial activity in agar medium using well diffusion assay by determining the zones of inhibition. The results showed fair growth in control conditions where no drug/extract was taken. Based on the zone of inhibition produced, *joshanda* proved to exhibit the good antibacterial activity which was quite comparable to the standard drug (ampicillin) reflected through inhibition zone of almost similar diameter (table 3).

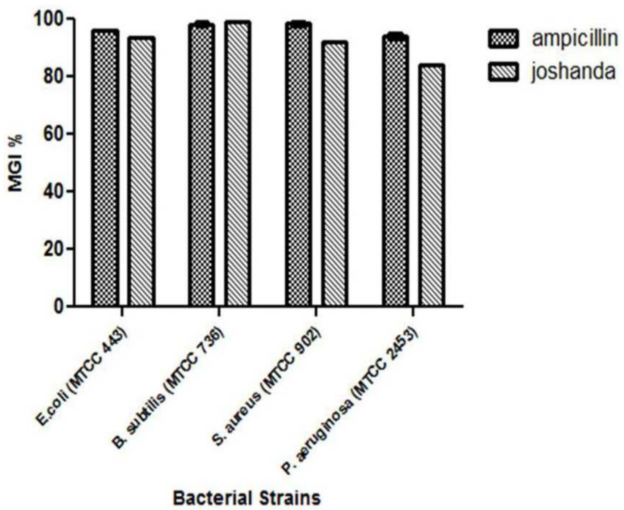

Fig. 1: Antibacterial activity of *joshanda* aqueous extract against *B. subtilis*, *S. aureus, E. coli* and *P. aeruginosa* by calculating % Mean Growth Inhibition, results represent the means±SEM from at least three separate experiments

Table 3: Zone of inhibition (mm) produced by control, ampicillin and joshanda aqueous extract when tested against *E. coli* and *S. aureus* expressed as mean±SEMrespectively

Components	Zone of inhibition (mm)	
	E. coli	*S. aureus*
Control	No zone of inhibition	No zone of inhibition
Ampicillin	25±0.851	24±0.921
Joshanda aqueous extract	17±0.798	15±0.826

Determination of antioxidant activity

DPPH assay

DPPH radical scavenging assay is the most extensively used method for screening antioxidant activity since it can furnish many samples in a short period and detect active ingredients at low concentration. The decrease in the absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It is visually notable as the color changes from purple to yellow. *Joshanda* aqueous extract showed DPPH radical scavenging activity in a concentration-dependent manner as shown in fig. 2. The highest and lowest scavenging was observed at amount 500 µl and 50 µl of the extracts. *Joshanda* extract demonstrated highest % DPPH radical scavenging of 98.379±0.313% and the minimum of 36.210±1.174%.

The results were quite comparable to that of standard i.e. ascorbic acid with maximum % DPPH scavenging of 98.843±0.443% and the minimum of 83.192±0.422%. The scavenging activity of *joshanda* with IC_{50} (inhibitory concentration) value of 114.49 µg/ml was quite comparable to the scavenging activity of ascorbic acid with IC_{50} value 60.55 µg/ml taken as standard.

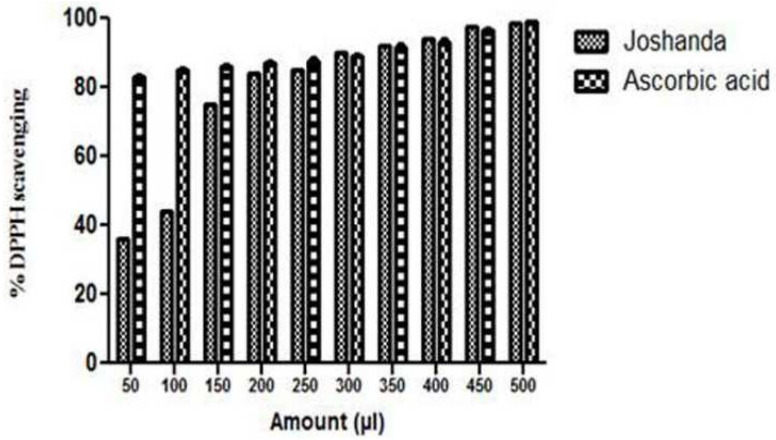

Fig. 2: DPPH free radical scavenging activity of *joshanda* were calculated and compared to ascorbic acid i.e. standard. The activity increased in time and concentration-dependent manner, results represent the mean±SEM from at least three separate experiments

CAT assay

Hydrogen peroxide (H_2O_2) is a non-radical reactive oxygen species with weak oxidizing activity. It diffuses through cell membranes rapidly and interacts with $Fe^{2+}$and possibly Cu^{2+}ions to form hydroxyl radicals and other free radicals. It is therefore biologically advantageous for cells to control the amount of H_2O_2 that is allowed to accumulate. The H_2O_2 scavenging ability of the aqueous extract of *joshanda* is shown in fig. 3.

The results show that *joshanda* exhibited significant H_2O_2 scavenging activity i.e. 0.047±0.001 µg/ml per minute degradation of H_2O_2 (fig. 3).

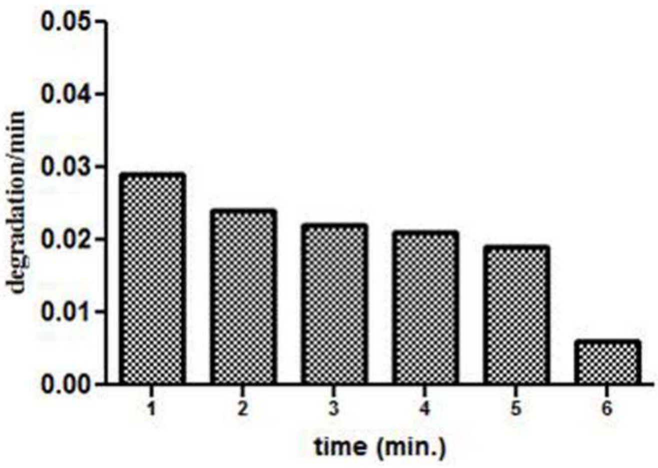

Fig. 3: H_2O_2 scavenging (%) was assessed with increasing amount of *joshanda* extract. The H_2O_2 degradation increased in concentration-dependent manner, results represent the means±SEM from at least three separate experiments

FRAP assay

The ferric reducing antioxidant power of *joshanda* is shown in fig. 4. The results showed that FRAP value of *joshanda* increase in the concentration-dependent manner. The highest absorbance of FRAP was observed in *joshanda* at 500 µl and the lowest was at 50µl with maximum FRAP value 0.945±0.024 mmol and the minimum value of 0.171±0.036 mmol respectively as compared to the standard which showed maximum FRAP value of 0.687±0.047 mmol and minimum at 0.059±0.005 mmol respectively. These concentrations were effective to react with ferric tripyridyltriazine (Fe III-TPTZ) complex and produce a blue colored ferrous tripyridyltriazine (Fe II-TPTZ). From the observations, it is clear that *joshanda* showed fair antioxidant activity comparable to ascorbic acid.

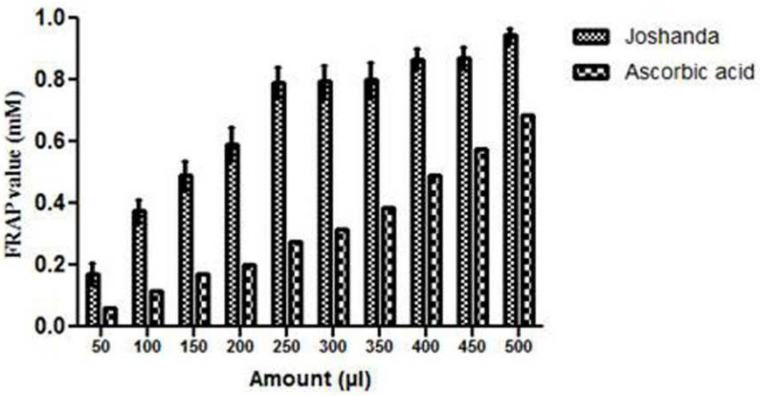

Fig. 4: FRAP value of *joshanda* extract was estimated and compared to the ascorbic acid as standard. The results were expressed in mmol, results represent the means±SEM from at least three separate experiments

Evaluation of anti-inflammatory activity of *joshanda*

Inhibition of albumin denaturation

Joshanda has mild anti-inflammatory property. If there occurs any infection or damage to the body or tissue then body show response against the infection through inflammation. Protein denaturation has to be one of the major causes of inflammation. Due to this, we have tried to find out the ability of *joshanda* to inhibit protein denaturation. The results showed that *joshanda* was effective in inhibiting thermally induced albumin denaturation at different concentrations (fig. 5). The *joshanda* extract showed the highest percentage inhibition of albumin denaturation of 14.069±0.350% and the lowest of 1.880±0.194% at extract volume or amount of 1000 µl and 100 µl respectively. The IC_{50} value of *joshanda* was found out to be 2.005 mg/ml. Aspirin which was used as standard drug showed the inhibition of 71±0.396% at 100 µg/ml.

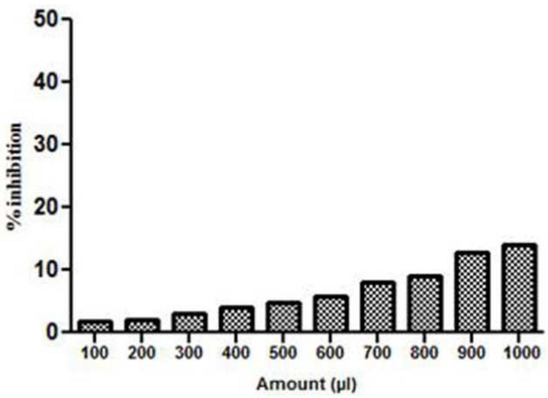

Fig. 5: Inhibition of albumin denaturation activity of *joshanda* was checked at various concentrations taking aspirin (100 µg/ml) as standard, results represent the means±SEM from at least three separate experiments

Proteinase inhibitory assay

The proteinase inhibitory assay showed that honey exhibits anti-inflammatory property in a concentration-dependent manner. Results exhibited significant anti-protease activity at different concentrations of *joshanda* extract. The extract demonstrated the highest percentage proteinase inhibition of 24.003±0.291 % and the lowest of 4.959±0.254% at extract volume or amount of 1000 µl and 100 µl respectively (fig. 6). Aspirin which was used as standard showed the maximum inhibition of 87±0.311% at 1000 µg/ml.

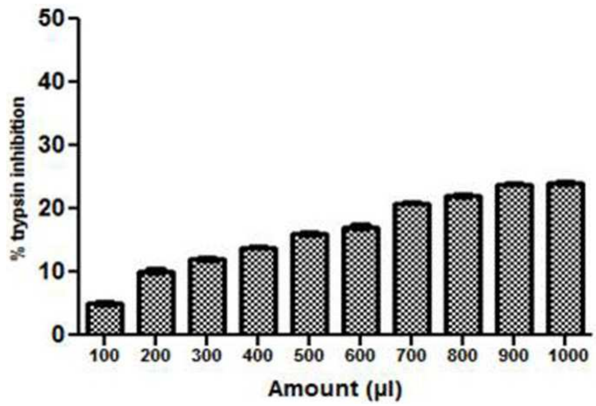

Fig. 6: % inhibition of trypsin activity in presence of *joshanda* extract was evaluated, results represent the means±SEM from at least three separate experiments

Determination of antifungal activity

Antifungal assay of the *joshanda* and its constituents extract was performed against *Candida* cells (ATCC 10261 and ATCC 90028). The extract showed no zone of inhibition up to 2 mg/ml as well as there was no inhibition in growth of *Candida* cells. Our results showed that *joshanda* had no potent anticandidal activity up to 1 mg/ml; increased concentration of extracts might lead the anticandidal effect.

DISCUSSION

The practice of traditional medicine (hikmath and homeopathy) is regulated by the Federal Government through Unani, Ayurvedic, and Homeopathic (UAH) Practitioners Act, 1965. In this regard, the National Council of Tibb (NCT) and National Council for Homeopathy (NCH) were established as corporate bodies under section 3 of the Act. Their formation was aimed to encourage and popularize the traditional system of medicine in masses [22, 23]. Therefore, in this study, *in vitro* pharmacotherapeutic properties and phytoconstituents of *joshanda* were assessed.

Secondary metabolite such as alkaloids, tannins, flavonoids are known to have activity against pathogens and therefore aid the antimicrobial activities of medicinal plants [24] which contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, anti-malarial, anti-cholinesterase, anti-leprosy activities etc [25-32]. From the present study, it is found that the decoction of *joshanda* contains flavonoids, alkaloid, tannin, phenol, glycosides, and terpenoids which are of great importance in the field of drug research. Therefore, further study must aim to isolate and purify them to confirm their pharmacological and medicinal use [33].

Protein denaturation and membrane leakage are the main cause of inflammatory processes implicated in the pathogenesis of diseases and infections. *Joshanda* was tested for their potential property of anti-inflammatory using two assays, Albumin denaturation, where the inhibition coagulation of protein by *joshanda* was tested and also protease inhibition where the inhibition of trypsin was tested. In both the cases, *joshanda* showed the minimal efficacy in both the reactions thus confirming lack the anti-inflammatory properties at low concentrations [34].

Antioxidants are very important since they possess the ability to protect the body from damage caused by free radical induced oxidative stress [35]. The antioxidant potential of joshanda was investigated in the search for affirmation of efficacy of this unani drug as decoction obtained from natural resources. It became clear that joshanda presents high antioxidant activity compared with reference antioxidant Vitamin C for DPPH scavenging activity and FRAP. Also, it showed fair activity when assessed by other methods. Hence, joshanda is a promising candidate for use as natural antioxidant for the health of human being.

Alkaloids and flavonoids are known to possess antibacterial activity. The antibacterial mechanisms of action of selected flavonoids are attributed to inhibition of DNA gyrase, cytoplasmic membrane function, and licochalcones A and C energy metabolism [36]. These phytochemical acts as natural antibiotics by preventing lipid peroxidation or by iron deprivation, hydrogen bonding, or specific interactions with vital proteins such as enzymes in microbial cells. *Joshanda*, being rich in alkaloids and flavonoids demonstrated antibacterial activity against four bacterial strains, two gram-positive and two gram-negative namely *B. subtilis* and *S. aureus* (gram positive) and *E. coli* and *P. aeruginosa* (gram-negative bacteria). The most significant finding in this study was that the heating treatment up to 100 °C could not impair the antibacterial action of the components. Till date, no antibiotic can withstand that heat treatment, to retain its antibacterial property. It would be of great significance if these heat stable and biologically active components are purified and characterized. The studies on purified antibacterial components and their cumulative action might indicate and elucidate their efficacy as future antibacterial remediation.

CONCLUSION

Joshanda was quantified for the main phytochemicals present in the extract. The presence of various phenolics and non-phenolics phytocompounds concluded that the plant might be of medicinal importance. The varying antioxidant (free radical scavenging) activities of extracts when compared to standard antioxidant i.e. Vitamin C, suggested the possibility that the antioxidant activity of this medicinal plant may contribute to play their role against various reactive oxygen species (ROS) mediated disorders such as cellular aging and cancer, becoming an alternative in the fight against skin aging and cancer cells. Altogether, these results establish the therapeutic applications of *joshanda* and its use as the herbal medicine for the prevention of inflammation and treatment of ROS and bacterial diseases. It can also be accomplished as an important mark in the field of human health and sciences. Finally, considering the results obtained, as future perspectives, we intend to evaluate some other biological activities, such as wound-healing, anti-malarial, antiviral, anti-cancer activity.

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CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest. It has not been published elsewhere. That it has not been simultaneously submitted for publication elsewhere. All authors agree to the submission to the journal.

AUTHOR CONTRIBUTION

Tooba Naz Shamsi: Concept, Data collection, and analysis, drafting article, writing manuscript.

Romana Parveen: Data collection and analysis, revision of the article, editing of the manuscript

Afaque Ahmad: Data collection, editing of the manuscript

Archoo Sajida: Sample and data collection, experimental work

Dr. Sadaf Fatima: Design of the work, data analysis, revision of the manuscript, final approval of the manuscript to be submitted for publication.

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