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Nutra-pharmaceutical efficacy appraisal of *Lavandula stoechas* leaves extracts in different solvents

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Present study was conducted to determine the antioxidant and antibacterial properties of *L. stoechas* leaves. Four solvents (80% methanol, 80% ethanol, 100% methanol and 100% ethanol) were used to extract the phytochemical, antifungal, and antioxidant components of *L. stoechas* leaves. In an 80% methanolic solvent, *L. stoechas* Leaves produced the highest extract yield (40.1 g/100g DW). Antioxidant examination of *L. stoechas* Leaves extracts in terms of total phenolic and total flavonoid contents revealed that the 80% methanolic extract had higher total phenolic content (33.2 mg GAE/g DW), as well as the higher total flavonoid content (46.5 mg CE/g DW. The DPPH radical scavenging activity and reduction power were used to assess antioxidant capacity. According to the findings, the 80% methanolic extract showed low potential and strong radical scavenging activity. The antimicrobial properties of *L. stoechas* leaves were determined by disc diffusion method. The antibacterial and antifungal potential of 80% methanolic extract against *E. coli* (30 mm DIZ) and *A. Paraciticus* (29 mm DIZ), respectively was observed. The results showed that 80% methanolic *L. stoechas* Leaves extract are potent source of nutra-pharmaceuticals agents for practical applications.

Capsule Summary: The antioxidant and antibacterial properties of *L. stoechas* leaves in four solvents (80% and 100% methanol and ethanol) were investigated and the extracts in different solvent system showed variable antimicrobial and antioxidant activities.

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INTRODUCTION

Lavadula stoechas belongs to a family Lamiaceae. Lavandula is derived from the Latin word 'violet' and 'to wash' and Stoechas comes from its cultivation on the Stoechades, a group of islands off the coast of Gaul near Massila, where it is commonly used by Muslim physicians. At the subcontinent, *L. stoechas* is called as "Ustukhuddus." It is incorrectly referred to as "Alfazema" in the West part of India. It is known in Spain as "Romero Santo" which means "holy rosemary."Unani describes it as "JaroobeDimagh" which stands for "breath of the brain," which enhances and improves intelligence due to the removal of black bile from the brain. This was first identified as "Galeenial herb" by Galen (first pharmacist). In the book "Kitab-ul-Hashaiash" Dioscorides mentioned it. In his well-known book, "The Canon of Medicine" Avicena (The Prince of Physicists)

mentioned it. *L. stoechas* oil percentage is between 0.77% and 1.2%. The best quality seedling is graded as gray and slightly bitter in taste with pungency. The flowers have the scent of Camphor. (Dar et al., 2012; Gupta et al., 2003; Husain et al., 1988; Jayaweera, 1980; Mahsud et al., 2010). Many historic civilizations in modern Egypt, China and India started to adopt herbal medicinal plants to cure diseases and that for spiritual reasons. (Alok et al., 2000; Bousta and Farah, 2020). While the practice of herbs for therapeutic reasons, dates back to the dawn of time, aromatherapy have experienced profound development in the 21st century, allowing the creation of an aromatherapy science (Esposito et al., 2014). The above herbal cure has been shown to be effective in treating disorders through using ingredients from medicinal herbs, like essential oils, hydrolic isolate, fruit drinks, and wax isolates. (Dunning, 2013; Steven and Ehrlich, 2009).

Upson (2002) revealed the synonyms of *Lavandula* L. include *Stoechas* Mill., *Fabricia Adans*, *Chaetostachys benth*, *Sabaudia buscal*, Muschl and *Isinia Rech*. The F. Lavandula is a genus of nearly 39 species, various hybrids, including 400 cultivars (Upson and Andrews, 2004). *L. angustifolia*, *L. stoechas*, *L. latifolia*, and the *L. intermedia* hybrid are quite well and commercially valuable varieties. Plant species in the Lavandula genus either subshrubs or perennial shrubs that can grow to be 1 meter tall. They choose acidic fields and flower during mid-June and mid-July. The Lavandula genus is known for its inflorescence. The flowers are organized in swirls and kept in clusters of tubular or quadrangular cymes, each with its own pedicel. Purple, blue, violet, or lilac are typical colors. The essential oils of *L. stoechas* were possibly one to be utilized. Their medicinal characteristics were known by the Romans, Greeks, and Arabs.(Siddiqui *et al.,* 2016).

Based on aforementioned facts, this study was conducted to determine the antioxidant and antibacterial properties of *L. stoechas* leaves. Four solvents (80% methanol, 80% ethanol, 100% methanol and 100% ethanol) were used to extract the phytochemical, antifungal, and antioxidant components of *L. stoechas* leaves.

MATERIAL AND METHODS

Chemicals, reagents and sample collection

Different chemicals were purchased from the Sigma Chemicals Co. (St, Louis, MO, USA). All standard antibiotic discs and culture media were purchased from Oxoid Ltd. (Hampshire, UK). Different parts of *L. stoechas,* i.e., leaves were obtained from the Lahore Garrison University, Lahore, Pakistan.

Pretreatment of plant sample

The leaves of *L. stoechas* were washed with tap water and then dried at 40 °C in an oven (Memmert, Jarmany) to maintain constant weight. By using a commercial blender, dried leaves were grounded into a fine powder. Then the ground material was passed through 80-mesh sieve. The passed material was used for extraction purpose. Polythene bags were used to store the ground samples at 4 °C until for further analysis.

Extraction procedure

For extraction of bioactive compounds, four solvent systems (100% methanol, 80% methanol, 100% ethanol and 80% ethanol) were being used. In this regard powdered leaves (20g) were extracted with 200mL in an orbital shaker for 8 hours at room temperature (Gallenkamp, UK). In order to separate the extract from residue by filtration. The resulting residues were extracted two times with the same solvent system. The drying of extracts was done at temperature of 45 ˚C and their yield was calculated by weighing extracts. The extracts were kept in a refrigerator at 4 ˚C for further analysis (Hassan, 2019).

Phytochemicals analysis

Total phenolic contents (TPC)

The method which was used to determine the Total phenolic contents of medicinal plant was based on the procedure of (Zafar et al., 2016) with some changes. Folin-Ciocalteu, a reagent that was used to determine the phenolic contents. 0.5 mL of Folin-Ciocalteu reagent was mixed with 50 mg of crude extract and then 7.5 mL deionized water was added in it. The mixture was retained at room temperature for 10 min and then 1.5 mL of 20% sodium carbonate (w/v) was added in it. The resulting mixture was kept in water bath for 20 min at temperature of 40˚C and then cooling of this mixture was done in an ice bath. By adjusting the spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan) at 755nm, absorbance was measured. Amount of TP was calculated by using gallic acid calibration curve. The results were expressed as mg GAE/g DW. Each sample was analyzed thrice to get average results of phenolics.

Total flavonoid contents (TFC)

The procedure of (Dewanto et al., 2002) was followed in order to determine the total flavonoid contents with minor changings. Aqueous extract of one milliliter that comprised of 0.01 g/mL of dry matter was placed in a volumetric flask having capacity of about 10 mL liquid. After that 5 mL of distilled water was added followed by 0.3 mL of 5% $NaNO_2$. After 5 min, 0.6 mL of 10% $AlCl_3$ was added in it. The solution was stayed for 5 min and then 2 mL of 1 M solution of NaOH was added and required volume was made up with distilled water. At 510 nm, absorbance was measured. Results of total flavonoids amounts were expressed as mg Catechin equivalent (CE)/g DW. Absorbance for each sample was taken three times in order to get the average results of total flavonoids.

Antioxidant activity

DPPH radical scavenging assay

DDPH radical scavenging assay was applied to determine the free radical scavenging activity of medicinal plants. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was used to determine the scavenging activity as described by (Rafique *et al.,* 2020) with little amendments. Methanol solvent was used to determine the DPPH solution (33 mg/L). Absorbance of the resulting solution was taken at 0 min. Then extract solutions (250µg/mL) were prepared. After that 5mL of methanolic solution of DPPH was added in 1mL of extract solution. The mixture was placed for 30 minutes in the dark place. Then by using a spectrophotometer, absorbance was measured at wavelength of 517nm with methanol was taken as a blank solution. Free radical scavenging activity was expressed as percentage inhibition and calculation was done by using Eq. 1 (Where, Ac and As are the absorbance values of control and sample, respectively).

$$\text{Inhibition } (\%) = \left[\frac{A_c - A_s}{A_c}\right] * 100 \qquad (1)$$

Determination of reducing power

With the minor changes to the approach given by (Hassan, 2019), the reduction power of plant seed extracts was determined. Different extracts with concentrations ranging from 2.5 to 10.0 mg were combined with a buffer consisting of sodium phosphate solution (5.0 mL, 0.2 M, pH 6.6) as well as potassium ferricyanide solution (5.0 mL, 1.0 percent). The resultant combination was kept warm for 20 minutes at fifty degrees Celsius. The blend was then centrifuged for 10 minutes at 980 g at 5 °C in a chilled centrifuge with 5 mL of 10% trichloroacetic acid included (CHM-17; Kokusan Denki, Tokyo, Japan). There were two layers created. The top layer of the solution (5 mL) was poured into a beaker but also diluted with 5.0 mL distilled water as well as ferric chloride (1.0 mL, 0.1 percent) solution, and absorbance has been measured at 700 nm with a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Each sample was analyzed three times and the findings were averaged.

Antimicrobial potential

Five fungal strains (*Aspergillus parasiticus, Aspergillus flavus, Fusarium oryzae, Fusarium tritichum, Aspergillus oryzae*) along with three strains of bacteria including *Escherichia coli*, *Pasturella multocida* and *Staphylococcus aureus* were taken through Fungal Bank, University of Punjab, Lahore, furthermore were tested individually against a panel The strains of bacteria were cultivated in nutritive agar at 37 °C, whereas the filamentous fungi were cultured in Potato Dextrose agar (Oxoid Hampshire, UK) nightly at 28 °C (Oxoid Hampshire, UK). Microbial strain slants were kept at 4 °C. The disc diffusion experiment was used to assess the antimicrobial properties of plant extracts.

Antimicrobial action of seeds of *L. stoechas was* tested against fungal strains (*Aspergillus parasiticus, Aspergillus flavus, Fusarium oryzae, Fusarium tritichum, Aspergillus oryzae*) and strains of *Staphylococcus*, *Escherichia coli* and *Pasturellamultocida aureus* by formerly approved technique (Bauer et al., 1966) with small tweaks. The potato dextrose agar (PDA) mix formed and inoculated. A sterile petri plate was filled with 20mL of PDA mixture and placed in a laminar air flow. Sterilised discs (6mm) of wicks sheet saturated with 50L of a meticulous plant extract were put on the agar plates. As positive references for fungal and bacterial isolates, correspondingly, fluconazol (30 g/disc) (Oxoid) and rifampicin (30 g/disc) (Oxoid) were used, to compare activity with conventional antibiotics. As a negative control, a disc with no samples was used. In separate petri plates, test discs and regular discs were inserted. For fungal growth, the plates were keep warmed at 280°C for 48 hours, and for bacterial growth, they were incubated at 370°C for 24 hours. The diameter of inhibition zones (mm) was measured by a zone reader to determine antimicrobial activity.

Statistical analysis

All of the studies have been done in triplicate (n=3), and the outcomes given as mean ±SD. Minitab 2000 Version 13.2 statistical software (Minitab Inc., Pennsylvania, USA) was used to analyze the data at a 5% significance level. The data on antifungal activity was provided as mean values with a 95% confidence range. LSD was used to calculate significant differences in mean.

RESULTS AND DISCUSSION

Percentage yield of extracts

The existence of antioxidant compounds with a variety of chemical characteristics and polarities, i.e., whether or not they are soluble in a given solvent, may be highly reliant on the solvent system utilized. Plant matrix polyphenols are also recovered using polar solvents. The most acceptable solvents include aqueous combinations of ethanol, methanol, acetone, and ethyl acetate. In order to isolate antioxidant substance from different plant plus food products, methanol and ethanol have been widely employed (Karaaslan Ayhan, 2021).

The use of methanol in antimicrobial (Vaghasiya and Chanda, 2007) and natural antioxidant components is considered to be a safer and widely-used solution 2007 (Anwar et al., 2010) Table 1 presents the extraction production from *L. stoechas* leaves in various solvent systems. In contrast, there was significantly higher ($p < 0.05$) extraction rate of 80% methanol from leaves (40.1 percent) went after by 80% ethanol > absolute methanol - absolute ethanol.

Total phenolic and flavonoid contents

Table 1: Yield, total phenolic contents, total flavonoids contents and DPPH radical scavenging activity

S. No	Solvents	Percentage yield (g/100g DW)	TPC (mg GAE/g DW)	TFC (mg CE/g DW)	DPPH (%) radical scavenging activity
1	80% Methanol	40.1±0.62^{a}	33.2±0.66^{a}	46.5±0.56^{a}	62.1±1.30^{a}
2	80% Ethanol	37.7±0.63^{b}	30.9±0.68^{b}	43.8±0.58^{ab}	60.9±0.55^{b}
3	100% Methanol	33.3±0.60^{c}	32.1±0.53^{ab}	41.7±0.43^{bc}	52.3±2.58^{bc}
4	100% Ethanol	32.2±0.65^{d}	29.3±0.55^{c}	39.2±0.35^{c}	50.4±0.75^{c}

Values are mean ± SD of four samples analyzed individually in triplicate at p <0.05. Superscripts alphabets within the column depicted significant difference among different solvent system.

Table 2: Reducing power of *l. stoechas* leaves

Solvent system	Concentration (mg/mL)			
	2.5^{a}	5.0^{b}	7.5^{c}	10^{d}
80% Methanol	0.123±0.03	0.129±0.02	0.137±0.01	0.150±0.06
80% ethanol	0.120±0.01	0.123±0.04	0.134±0.03	0.142±0.05
100% ethanol	0.115±0.06	0.117±0.02	0.125±0.01	0.136±0.06
100% methanol	0.118±0.04	0.128±0.01	0.131±0.06	0.137±0.02

Explanation as given in Table 1

These compounds are considered the largest antioxidant in plants. Polyphenolic compounds with oxidative enzyme inhibition, free radical scavenging activity, anti-inflammatory activity, and hydrolytic enzyme inhibition are among the features of these components (Atanassova et al., 2011). Effective antioxidative characteristics are responsible for the majority of their activities (Soares et al., 2020).

The total phenolic concentrations increase sharply with the increasing fruit size. To assess the overall phenolic content of natural products, the Folin-Ciocalteu method is used (Zhang et al., 2020). The oxidation and reduction reactions are the basis of its mechanism (Novakov *et al.,* 2021). The total phenol content in *L. stoechas* leaves was significantly ($p < 0.05$) and varied from 29.3-33.2 (mg GAE/g DW) given below in Table 1. It has been found out that 80% methanol extract showed highest TPCs, follow up by: absolute methanol > 80% ethanol > absolute ethanol, obtained maximum TPC from leaves. It was found that 80% methanol solvent method, follow up by: 80% ethanol > absolute, extracted.

The current results are consistent with those of (Ahiakpa et al., 2013), who discovered that flavonoids in *L. stoechas* pods were greater in methanolic extracts than in aqueous and other extracts, suggesting that the ethanolic extraction strategy was more successful. It was hypothesized that the flavonoids in *L. stoechas* pods are more soluble in organic solvents than in aqueous solvents. Furthermore, in an aqueous methanolic extract of P. pinnata, (Sajid et al., 2012) looked at the total flavonoids concentration (24.6 mg/100 g quercetin equivalent).

Antioxidant activity

DPPH radical scavenging activity

DDPH-free radical scavenging potential has also been shown to raise the extract concentration (Ebrahimzadeh et al., 2010). Thus, a free radical scavenging process based on the phenomenon of electron transfer is used in DPPH radical. This is a test of antioxidant that produces a violet coloration in the solution of methanol. The stability of free radical is decreased at room temperature due to the existence of antioxidant molecule, which produces a solution without color (Garcia *et al.,* 2012). The oxidation process of the radical proton scavenger is an important mechanism. By reducing the absorption of a DPPH solution to 517 nm, its ability to reduce the activity of the plant extract by antioxidant has been evaluated (Chougule et al., 2012) The antioxidant molecule has the atom that gives hydrogen that gives to its free radical breaking-up that is a significant antioxidant value (Aliyu et al., 2009). The DDPH-free radical savaging test was used to determine the antioxidant capacity of medicinal plants *L. stoechas*. This test investigates the possible new natural antioxidant sources. The DPPH concentration was measured and found to be reduced due to scavenging ability of medicinal herb leaves. Table 1 demonstrated that the radical DPPH scavenging behaviors of A are substantially distinguished. Extracts of esculentus in the various solvent system. The liquid extracts of the *L. stoechas* leaves had a radical capacity to scavenge radically DDPH. In 80% of the methanol extract, 80% ethanol > absolute andhanole was followed in the *L. stoechas leaves*, which showed the highest DDPH radical breakage potential.

Reducing power

The reduction in plant extract capabilities may indicate their capacity for antioxidant activity (Zhang et al., 2011). Examination of medicine plants or plants shows that plants are the main source of antioxidants. These plants can apply

protective effects to certain oxidative stresses in biological systems (Sylvie et al., 2014). Reducing medicinal plant ability was of higher absorption. Activity of power reduction therefore increases with increased extract concentration (Nishaa *et al.,* 2012). Table 2 explains the reducing ability of the leaves of *L. stoechas.* Reducing Potential increases in concentrations from 2.5 to 10.0 mg/mL for the isolates were observed. However, the largest power reduction was demonstrated by 80% methanol leaven extract. As reducing power is dependent on concentration so the given results disclosed that the reducing power of *L. stoechas* is enhanced with increase in concentration.

extract was applied individually against bacterial stresses, and the zone reader was used to calculate the inhibition zone diameter (DIZ). The findings showed the highest inhibitor activity of the methanol leaves extract against *P. multocida* ($p < 0.05$) of all solvents (30 mm DIZ).

Antifungal activity

The revealing method against a panel of fine strain (*Aspergillus parasiticus, Aspergillus flavus, Fusarium oryzae, Fusarium tritichum, Aspergillus oryzae*) investigated the antifungal properties of *L. stoechas* feeding stuffs and semen. Table 4 shows the diameter of the inhibition zones.

Table 3: Antibacterial activity of L. *stoechas* leaves extracts

Microorganism	DIZ (mm)			
	80% Ethanol	Absolute Ethanol	80% Methanol	Absolute Methanol
E. coli	$29\pm1.26^{ab}{}_{b}$	$27\pm1.30^{bc}{}_{bc}$	$30\pm1.69^{a}{}_{b}$	$22\pm251^{c}{}_{bc}$
P. multocida	$23\pm1.89^{c}{}_{c}$	$29\pm2.18^{b}{}_{b}$	$32\pm1.88^{a}{}_{bc}$	$26\pm1.99^{d}{}_{b}$
S. aureus	$17\pm1.34^{ab}{}_{d}$	$14\pm1.66^{c}{}_{c}$	$20\pm1.57^{a}{}_{c}$	$15\pm2.42^{bc}{}_{c}$

Values are mean±SD of three samples analyzed individually in triplicate at $p < 0.05$. Superscripts within the same column depicted significant difference among different solvents, while subscripts within the same row indicated significant difference ($p < 0.05$) between bacterial strains.

Table 4: Antifungal activity of *L. stoechas* leaves extracts

Microorganisms	DIZ (mm)			
	80% methanol	80% ethanol	100% methanol	100% ethanol
A. parasiticus	$31\pm1.14_{a}{}^{a}$	$23\pm1.77^{bc}{}_{bc}$	$26\pm1.43^{ab}{}_{ab}$	$21\pm2.10^{c}{}_{c}$
A. flavus	$27\pm1.29^{ab}{}_{b}$	$24\pm1.68^{a}{}_{ab}$	$21\pm2.06^{c}{}_{c}$	$22\pm1.83^{b}{}_{bc}$
F. oryzae	Nil	Nil	Nil	Nil
F. tritichum	$21\pm2.08^{c}{}_{ab}$	$17\pm1.89^{c}{}_{c}$	$24\pm2.05^{ab}{}_{a}$	$19\pm2.09^{c}{}_{b}$
A. oryzae	$22\pm1.10^{b}{}_{a}$	$17\pm1.56^{c}{}_{b}$	$15\pm1.59^{d}{}_{c}$	$15\pm2.09^{d}{}_{c}$
Flucanozole	$19\pm1.10^{a}{}_{b}$	$18\pm1.46^{a}{}_{c}$	$19\pm1.36^{a}{}_{a}$	$14\pm1.67^{a}{}_{d}$

Explanation as given in Table 3

Antimicrobial activity

Antibacterial activity

A variety of medicinal plants can treat many infections and they carry potential antimicrobial activity (Javid *et al.,* 2015). The phytochemical study of *L. stoechas* methanol extract revealed that certain phytoconstituents including alkaloids, saponins, cardenolides, anthraquinones and tannis exist in the methanol extract. The antimicrobial ability of these plant metabolites has been defined. The presence of these secondary metabolites may thus attribute antimicrobial properties of the medicinal plants.

In any plant area such as leaves, fruits, roots, flowers, pods and stems, the antibacterial components can exist (Banerji et al., 2021). During this investigation, a method of diffusion was used to assess the behavior antibacterial to three strains of bacteria: *P. Multocida, E. coli and S. aureus* of the leave extracts of the *L. stoechas*. Each

Results showed that the highest inhibitory activity (29 mm DIZ) was found to be 80% methanolic extracts of *L. stoechas leaves* ($p < 0.05$), against *A. parasiticus*. Extracts of ethanol leaves demonstrated modest activity against fungal strains. Based on the findings, leaves also showed no activity against *Fusarium oryzae* activity. The findings (Dahham et al., 2010) investigate that *Punica granatum* methanolic extracts had greater effect on fungal strains that could be attributed because of the existence of some active phytoconstituents, including phenols, flavonoids and tannins. Natural products from conventional medicinal plants have often opened the way to modern therapeutic forms. Plants are the primary source of drugs among all of these. Herbal medicine occupies a unique position in the pharmaceutical industry. In Greek literature, *L. stoechas* has been identified as having antiseptic, anticonvulsant, numbness, shaking, mania, amnesia, demulcent, phlegmagogue, nervine tonic, antianxiety and pharmacological. The phytochemistry, pharmacognostic

and pharmacological activities of *L. stoechas* are is assessed in this study and results revealed that *L. stoechas* has potential applications for antioxidant and antimicrobial formulations.

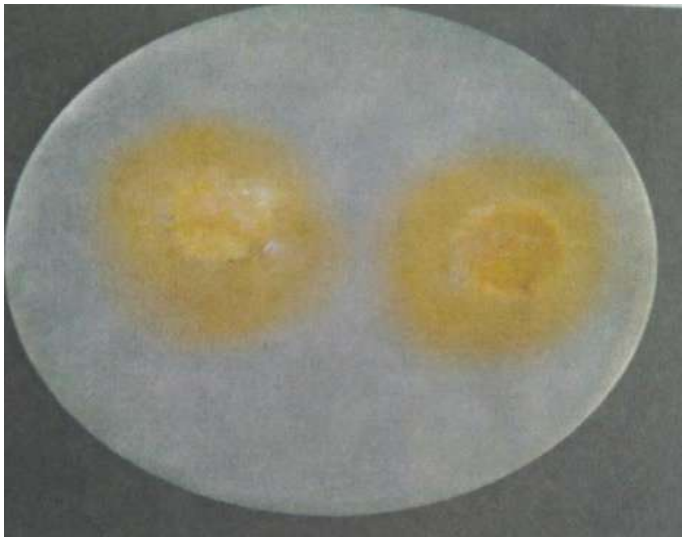

Fig. 1: Zone of inhibition against *A. flavus* for 80% and absolute ethanolic of *L. stoechas*

CONCLUSIONS

This present study examined the biological activities of the *L. stoechas* extract along with phytoconstituents. A significant amount of TFC was recorded in the extracts of *L. stoechas*. The extracts of *L. stoechas* showed promising antioxidant activity. The *L. stoechas* extracts exhibited powerful antimicrobial activity. In view of promising bioactivities, the *L. stoechas* can be used in preparation of formulations for food, cosmetics and pharmaceutical industries, which is a natural source of bioactive agents responsible for bioactivities.

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