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Original Research Article

EVALUATION OF ANTIMICROBIAL PROPERTIES, TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENTS OF PLANT EXTRACTS *LAVANDULA STOECHAS* LINN

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Abstract: *Lavandula Stoechas* is a Plant of the Lamiaceae/Labiatae family. The aim of this study was to screen the Phytochemicals, to evaluate the total flavonoid and total Phenolic contents as well as an Antimicrobial activity of hydroalcoholic extract of *Lavandula Stoechas.* Plant material was extracted by using hydroalcoholic solvent for 2 h and repeated 3 times. Total flavonoid content was determined by aluminium chloride colorimetric assay on 420 nm. Total Phenolic content was determined with Folin-Ciocalteu 1:4 on 765 nm using microplate reader. Phytochemical screening showed that all of samples positively contain alkaloid, glycoside, flavonoid, phenolics, carbohydrate, diterpenes and saponin. Total flavonoid content was found to be 9.31(QEmg/100mg) where as total Phenols was found to be 5.52 (GAEmg/100mg). Which could be related to its higher Phenolic content. It can be hypothesised that the high contents of Phenolic compounds of *Lavandula Stoechas* indicated that these compounds contribute to the Antimicrobial activity and can be regarded as promising plant species for natural sources of Antimicrobial activity with potential value for treatment of many life threatening diseases.

Key words: *Lavandula Stoechas* Linn, Phytochemical Screening, Antimicrobial, Total Flavonoid, Total Phenolic content.

Introduction: Herbal medicines play a major role in primary health care, mainly in the developing countries. Therapeutic potential of herbal drugs are attributed to the present of bioactive Phytochemicals. Plants are biosynthetic laboratories of a wide spectrum of chemicals of various physiological functions. These phytochemicals are believed to have better compatibility with the human body and possess medicinal properties. Herbal drugs got a successful history as old as human civilization and today herbal medicines are coming back into prominence because of decreasing efficacy and serious side effects of the modern medicines.

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Lavandula Stoechas belongs to the family Labiatae (Lamiacae) and have been used in dried form for centuries for a variety of therapeutic and cosmetic purposes, including antibacterial, antifungal and anti-depressive uses with over 150 active constituents, including camphor, linalool, linalyl acetate, 1,8-cineole, β-cymene and terpinen-4-ol as the main components [2].The medicinal importance of the plant is well documented [3-5] and the drugs prepared from this plant are registered in many Pharmacopeia. The plant is used as expectorant, antispasmodic, carminative, a good stimulant, deobstruent, resoluent and wound healing. The essential oil obtained from its flowering twigs has been used as a remedy against colic and chest affections, to relieve nervous headache, biliousness and for cleansing wounds [6-8].

In particular, despite widespread of these plants, the literature contains few reports of antioxidant activity and chemical composition of these plants. In present study, we carried out a systematic record of chemical composition and the antimicrobial activity through determination of total phenolics and flavonoids content, as well as antimicrobial activity of hydroalcoholic extract of *Lavandula Stoechas.*

Material and Methods

Chemical and reagents: All the chemicals and reagents used in the study were of analytical grade.

Selection, Collection and Authentication of Plant/Plant Material: The *Lavandula Stoechas* Were Collected in the Months November 2017 to January 2018 from the Vindhya herbal garden, Bhopal M.P. and identified & authenticated by Dr. Zia Ul Hasan, Professor, Head Dept. of Botany, Safia College of Science, Bhopal, M.P., dated 22/12/2017. M.P. and were deposited as herbarium with voucher specimen No. 470/Bot/Safia.

Extraction: The Plant material was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with Petroleum ether (60-80°C) in a Soxhlet apparatus. The extraction was continued till the defatting of the material had taken place. The air-dried and powdered defatted marc of the plant material was subjected to extraction with hydroalcoholic solvent in a soxhlet apparatus and concentrated at 40°C with a rotary evaporator.

Preliminary Phytochemical Screening of Extract: Phytochemical Screening of the hydroalcoholic extract was Performed to investigate the presence or absence of the different phytochemical constituents such as phenols, flavonoids, saponins, tannins, steroids, terpenoids, coumarins, cardiac glycosides etc. using standard procedures [9-12].

Determination of total flavonoid content: Total flavonoid content was determined by aluminium chloride colorimetric assay adapted from Chatatikunet at [15] and Sandip *et al* [16] with slight modification.10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 25- 125µg/ml were prepared in methanol.10 mg extract dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoid.1 ml of 2% $AlCl_3$methanolic solution was added to 3 ml of extract or standard and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm.

Determination of total Phenolic content: The total phenolic content of the extract will be done by the modified Folin- Ciocalteu method [17]. 50 mg gallic acid was dissolved in 50 ml methanol, various aliquots of 25- 125µg/ml was prepared in methanol.10 mg extract dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol.2 ml of extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (75g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Antibacterial Activity of Phytochemical Extracts

Microbial cultures: The studies of antimicrobial effect of Phytochemical obtained from ***Lavandula stoechas*** extract a medicinally important plant, there were 3, microbial successfully procured from Microbial Culture collection, National Centre for cell science, Pune, Maharashtra, India The lyophilized cultures of bacterial strains upon culturing in nutrient broth for 24-48 hours at 37^{o}C in an incubator resulted into turbid suspension of activated live bacterial cell ready to be used for microbiological study. The list of bacterial species with suitable codes used in the antimicrobial studies is given in the table. From the broth of respective revived cultures of bacteria loop full of inoculum is taken and streaked on to the nutrient agar medium and incubated again at same culture conditions and duration that yielded the pure culture colonies on to the surface of the agar culture that are successfully stored in refrigerated conditions at 4^{o}C as stock culture to be used for further experimentation.

Antimicrobial Studies: The lawn cultures were prepare with all the microbes used under present study and sensitivity of microbes towards the various Phytochemical extracts obtained from the ***Lavandula stoechas*** were studied at the concentration of 100 mg/ml using well diffusion method.

Results and Discussion: Phytochemicals are the core of phytomedicines; their therapeutic efficiency directly correlates with the presence of various phytochemicals. Table 1shows the phytochemical screening of hydroalcoholic extract. Alkaloids, Flavonoids, glycosides, diterpenes**,** phenolics, and saponins were present in hydroalcoholicextract.

Preliminary phytochemical screening experiments are commonly performed to promote a guidance of substantial phytochemicals that may be involved in the antioxidant activity of plant extracts [19-21].

Table 1: Phytochemical Screening of hydroalcoholic extract of *Lavandula Stoechas*

S. No.	Constituents	*Lavandula Stoechas*
1.	Alkaloids	Positive
2.	Glycosides	Positive
3.	Flavonoids	Positive
4.	Phenolics	Positive
5.	Amino Acids	Negative
6.	Carbohydrate	Positive
7.	Proteins	Negative
8.	Saponins	Positive
9.	Diterpines	Positive

Table 2: Thin layer chromatography of extract

S. No.	Mobile Phase	Extract	Rf value
1.	Toluene : Ethyl Acetate : Formic acid (Phenol)(7:5:1 v/v)	Hydroalcoholic	0.516
2.	Toluene : Ethyl Acetate : Formic acid (Flavonoid)(5:4:1 v/v)	Hydroalcoholic	0.836

Phenolic compounds are considered important natural antioxidants and represent one of the most abundant compounds in plants. They display several functions such as pigmentation, protection against ultraviolet rays, allelopathic action, defense against microbial attack and predators. Among the polyphenol compounds, the most studied subclass is the flavonoids which in plants are commonly found conjugated to sugars. The total flavonoid content was quantified by the aluminum chloride method and expressed as quercetin equivalents (QE) per gram of substrate and the total phenolic content was quantified by the Folin- Ciocalteu method and expressed as gallic acid equivalents (GAE) per gram of substrate.

Table 3: Preparation of calibration curve of gallic acid

S. No.	Concentration (µg/ml)	Absorbance λmax=760 nm
0	0	0
1	25	0.356
2	50	0.605
3	75	0.854
4	100	1.15
5	125	1.351

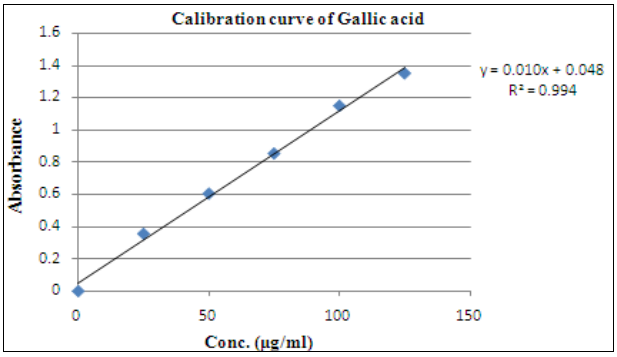

Figure 1: Calibration curve of gallic acid

Table 4: Preparation of calibration curve of quercetin

S. No.	Concentration (µg/ml)	Absorbance λmax=420 nm
0	0	0
1	25	0.234
2	50	0.448
3	75	0.658
4	100	0.869
5	125	1.102

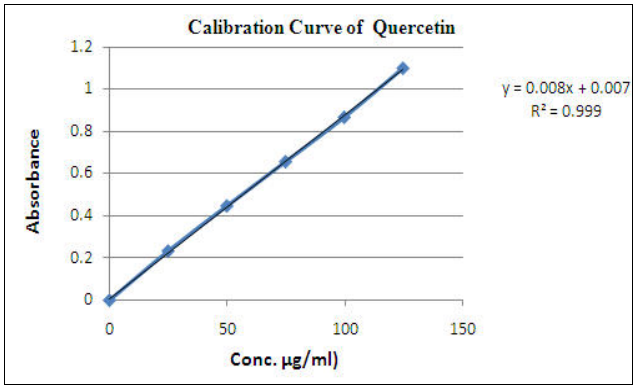

Figure 2: Calibration curve of quercetin

The content of total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y =0.010x + 0.048, R^2= 0.994, where × is the gallicacid equivalent (GAE) and y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: Y=0.008 X+0.007, R^2=0.999, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 5 shows the total phenolic and total flavonoid content estimation. Total flavonoid content was found to be 9.31(QEmg/100mg) whereas total phenols was found to be 5.52 (GAEmg/100mg).

Table 5: Total Phenolic and flavonoid content

S. No.	Hydroalcoholic extract	Total Phenol (GAE) (mg/100mg)	Total Flavonoid(QE) (mg/100mg)
1.	*Lavandulastoechas*	5.52	9.31

Table No. 6: Results of antibiotic sensitivity of phytochemical extract

S.N	Codes Bacteria	Bacterial Strains	Activity
1.	Bact-1	*E. Coli*	Yes
2.	Bact-2	*S. Mutans*	Yes
3.	Fungus	*Candida albicans*	Yes

Antibiogram studies: The present investigation in this research work, the antimicrobial activity of extract obtained from the plant aerial part was evaluated against bacterial and fungal pathogens used under present study. The fresh pure 100% extracts obtained from plant used to suitably dilute upto the concentrations of 100, 50 and 25 mg per ml and applied on to the test organism using well diffusion method. Results of the experiment are being concluded in the Table, which clearly shows the anti-microbial activity of extract of ***Lavandula stoechas*** **out** of the 2 bacterial strains and 1 Fungus used in present work.

Table No.7: Antimicrobial activity of Plant extract on different antimicrobial agents

S.N	Name of drug	Microbes	Zone of inhibition		
			10 µg/ml	**20 µg/ml**	**30 µg/ml**
1	Ciprofloxacin	*E. Coli*	9±0.15	13±0.13	16±0.19
		S. Mutans	16±0.14	20±0.18	25±0.15
2	Fluconazole	*Candida albicans*	8±0.11	12±0.09	14±0.04

Table No.8: Antimicrobial activity of Plant extract on different microbes

S. No.	Plant	Name of microbes	Zone of inhibition		
			25mg/ml	**50 mg/ml**	**100mg/ml**
1.	***Lavandula stoechas***	*E. Coli*	8±0.12	14±0.15	15±0.11
		S. Mutans	10±0.14	14±0.41	18±0.12
		Candida albicans	7±0.23	8±0.21	17±0.08

Photo plates of Antimicrobial Study *Lavandula stoechas* hydro alcoholic extracts on Different bacteria

Figure No. 1: Photoplates of Antbacterial activity of Ciprofloxacin and *Lavandula stoechas* hydro alcoholic extract on *E. Coli*

Figure No.2: Photoplates of Antbacterial activity of Ciprofloxacin and *Lavandula stoechas* hydro alcoholic extract on *S. Mutans*

Figure No.3: Photoplates of Antifungal activity of Fluconazole and *Lavandula stoechas* hydro alcoholic extract on *Candida albicans*

Conclusion: Based on the results obtained in the present study, it is concluded that the hydroalcoholic extract of *Lavandula stoechas* exhibit considerable Antbacterial activity and they possess substantial amounts of phenolic compounds. This study revealed that the *Lavandula stoechas* contain appreciable amounts of polyphenolic compounds that are capable of eliciting potent Antbacterial activity. This study revealed that the *Lavandula stoechas* contain appreciable amounts of polyphenolic compounds that are capable of eliciting potent Antbacterial activity. Thus, hydroalcoholic extract can be considered a good source of Antbacterial which might be beneficial for combating oxidative stress. Thus indicating the key role that phenolic compounds may exert on the Antbacterial activity of these plants. Hence more studies are required to isolate and identify these bioactive compounds responsible for such

activities so as to assess their Antbacterial activity *in vivo*.

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