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PHYTOCHEMICAL SCREENING, TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF *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 antioxidant 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. Antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenger methods.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) whereas total Phenols was found to be 5.52 (GAEmg/100mg). The hydroalcoholic extract displayed DPPH free radical scavenging activity with76.41±0.47IC50 value, 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 antioxidant activity and can be regarded as promising plant species for natural sources of radical scavenging activity with potential value for treatment of many life threatening diseases.

Keywords: *Lavandula Stoechas* Linn, Phytochemical Screening, DPPH, 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. Oxidation is necessary for energy production in all living systems. However it can produce free radicals, which can start chain reactions that may damage cells. Antioxidants terminate these chain reactions by removing radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. Generally, antioxidants of plant origin are of often reducing agents such as thiols or polyphenols. [1]

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, bliousness 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 antioxidant activity through determination of total phenolics and flavonoids content, as well as DPPH radical scavenging 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]

Identification of bioactive compound by TLC

Identification of bioactive compound was performed by thinlayer chromatography (TLC) on silica plates (60F254, aluminum backed, 200 µm layer thickness, 10.0 x 5.0 cm). The presence of flavonoids, phenylpropanoids, alkaloids, terpenes, steroids, coumarins, quinones and proanthocyanidins were investigated using adequate development systems and revealers[12-14]. After development, the plates were air dried and sprayed with the revealers in a fume hood.

Determination of total flavonoid content

Total flavonoid content was determined by aluminium chloride colorimetric assay adapted from Chatatikunet et al., [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]. 10 mg gallic acid was dissolved in 10 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 (7.5g/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.

***In-vitro* free radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl - DPPH)**

DPPH scavenging activity was measured by the spectrophotometer[18]. Stock solution (1.5 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml of methanol gave an initial absorbance of 0.886. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 100 mg of the extract was dissolved in 100 ml of methanol to get 1000 µg/ml solution to prepare stock solution of test sample. 10, 20, 40, 60, 80 and 100µg/ml solution of the test samples were prepared from stock solution. 15 mg of DPPH was dissolved in 10 ml of methanol. The final solution was covered with aluminum foil to protect from light. 75 µl of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75 µl of DPPH and 50 µl of the test sample of different concentration were put in a series of

volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken.Absorbance at zero time was taken for each concentration.Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

Calculation of % reduction=Control Absorbance-Test absorbance/Control Absorbance x 100

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 hydroalcoholic extract.

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

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 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 theFolin- Ciocalteu methodand 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

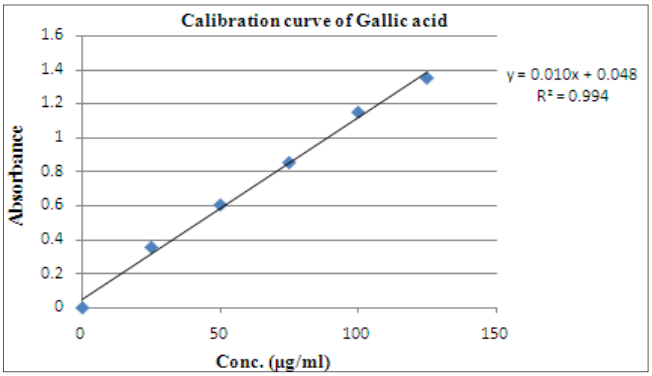

Figure 1: Calibration curve of gallic acid

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

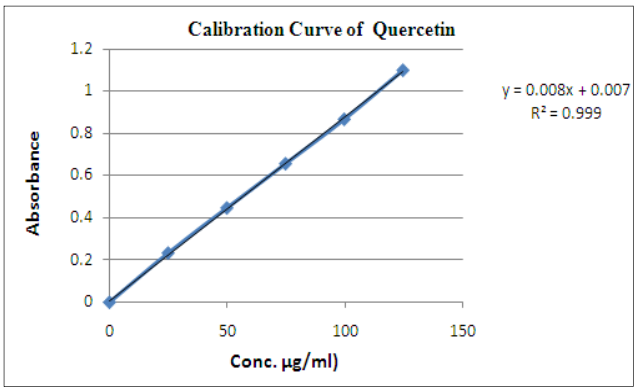

Figure 2: Calibration curve of quercetin

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 gallic acid 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: 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 5 shows the total phenolic and total flavonoid content estimation. Total flavonoid content was found to be 9.31(QE mg/100mg) whereas total phenols was found to be 5.52 (GAE mg/100mg).

Table 6: DPPH radical scavenging activity

S. No.	Concentration (µg/ml)	Vitamin C		Hydro alcoholic extract	
		Absorbance	% Inhibition	Absorbance	% Inhibition
1	10	0.669	24.49±0.22	0.834	5.86±0.49
2	20	0.469	47.06±0.37	0.769	13.2±0.74
3	40	0.234	73.58±0.28	0.683	22.91±0.68
4	60	0.106	88.03±0.19	0.524	40.85±0.24
5	80	0.051	94.24±0.14	0.406	54.17±0.66
6	100	0.046	94.8± 0.26	0.308	65.23±0.52
	IC 50 Value		**21.7±0.36**		**76.41±0.47**

Absorbance of 0.01mM DPPH Solution =0.886

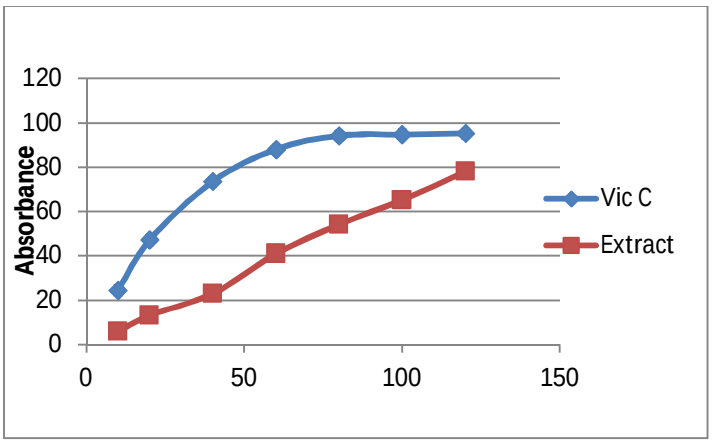

Figure3: Graph of *in vitro* free radical scavenging activity

The hydroalcoholic extract displayed DPPH free radical scavenging activity with 76.41±0.47 IC_{50} value, which could be related to its higher phenolic content. High total phenol and flavonoid contents of plant may be a reason for its higher DPPH-scavenging activity, supporting the opinion that plant extract have a potent antioxidant activity mainly due to their richness of phenolic compounds. This study revealed that the *Lavandula stoechas* contain appreciable amounts of polyphenolic compounds that are capable of eliciting potent antioxidant activities. The antioxidant profile of this plant can be harnessed to treat radicals related to pathological conditions. Further studies are needed for the isolation and elucidation of the structure of these components and also more investigations are necessary for better understanding of their mechanism of action as antioxidants.

CONCLUSION

Based on the results obtained in the present study, it is concluded that the hydroalcoholic extract of *Lavandula stoechas* exhibit considerable antioxidant radical scavenging activity and they possess substantial amounts of phenolic compounds. Thus, hydroalcoholic extract can be considered a good source of antioxidants which might be beneficial for combating oxidative stress. A positive correlation was found between total phenolic content and radical scavenging activity, thus indicating the key role that phenolic compounds may exert on the antioxidant 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 antioxidant activity *in vivo*.

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