



Phytochemical, antioxidant and antimicrobial studies of *Salvia splendens* leaves

Mahendra Pratap Chopra*, Papiya Mitra Mazumder, Dinakar Sasmal
and Sanjeeb Kumar Patro

Department of Pharmaceutical Sciences & Technology, Birla Institute of Technology, Mesra, Ranchi

ABSTRACT

Plants are the reservoir of effective chemotherapeutics and can provide valuable sources of natural antimicrobials. *S. splendens* plant has been used in traditional medicine as an anti-inflammatory, anti diabetic and antibacterial agent. The aim of this research was to evaluate the phytochemical together with antioxidant, and antimicrobial activities of *S. splendens* leaves. The phytochemical constituents of the leaves were extracted by successive extraction using soxhlet apparatus. Qualitative and quantitative phytochemical analysis of extracts and fractions were carried out. The Ethyl acetate fraction of methanolic extract of *S. splendens* leaves was selected for antioxidant and antimicrobial activities. Antioxidant status was evaluated using DPPH Scavenging activity and Hydroxyl radical scavenging activity methods. The total antioxidant capacity was determined in terms of GAE (gallic acid equivalents). Antimicrobial activity was carried out by method described by Ver-poorte. The phytochemical screening showed positive results for triterpenes, steroids, alkaloids, anthraquinones, flavonoids, saponins, tannins, and phenolic acids. The methanolic extract was found to contain highest flavonoid content of 117.4 mg Quercetin equivalent per 1gm and phenolic content of 17 mg of gallic acid equivalent per 1gm respectively and showed highest percentage inhibitory activity for antioxidants. The *S. splendens* leaves exhibited significant antioxidant properties, in addition to antimicrobial effects against the selected microorganisms..

Key words: *Salvia splendens*, Phytochemical, *in vitro* antioxidant, ethyl acetate fraction, minimum inhibitory concentration (MIC)

INTRODUCTION

The genus *Salvia* (commonly known as sage) is a broad genus belonging to the family Lamiaceae which is a large cosmopolitan family of approximately 252 genera and 7,200 species [1, 2]. Several species of *Salvia* are cultivated for their aromatic characteristics and are used as flavorings, food condiments, cosmetics and perfume additives [3]. Additionally, *Salvia* species have been widely used in folk medicines as antibacterial, antitumor, antioxidant and anti-inflammatory activity besides diabetes, arthritis, headache and diuretic [4, 5].

Phytochemical studies conducted on plants of this genus have led to the isolation of numerous diterpenoids [6,7,8]. Triterpenes and sterols were also reported [9], in addition to anthocyanins, flavonoids, phenolic acids and their derivatives [4]. Several studies have investigated the antioxidant activities of *Salvia* species [5,10-13], including screening of the antioxidant potential of some *Salvia* extracts. Moreover, the anti-inflammatory [13,14], and antimicrobial effects [5, 15-19] of different *Salvia* species have also been studied. Plant drugs are frequently considered to be less toxic and free of side effects than synthetic ones. *Salvia* is an important genus widely cultivated and used in flavoring and folk medicines. *Salvia* species are used as traditional medicines all around the world, possessing antibacterial, antioxidant, anti-inflammatory and analgesic properties.

The aim of this study was to evaluate Phytochemical, antioxidant and antimicrobial activities. of leaves of *S. splendens*.

EXPERIMENTAL SECTION

Plant material

Leaves of *S. Splendens* were collected from the campus of Birla Institute of Technology, Mesra, Ranchi. The plant was identified and authenticated by taxonomy department of Botanical Survey of India (BSI), Kolkata. The voucher specimen (CNH/76/2012/Tech.II/898) was retained in the Department of Pharm. Sciences & Technology, BIT-Mesra, Ranchi, Jharkhand (India) for future reference.

Preparation of plant extracts

The *S. splendens* leaves were collected and washed to remove the adhered debris. It was dried in shade at room temperature. The dried leaves were coarsely powdered using tissue blender. The air dried powder was successively extracted by hot extraction process using Soxhlet apparatus with solvents of increasing polarity index viz., petroleum ether (60-80 grade), chloroform and methanol for 72 hours. After filtering the extracts, the filtrates were dried using rotary-evaporator to get dried crude fractional extracts [20].

Results**Phytochemical screening**

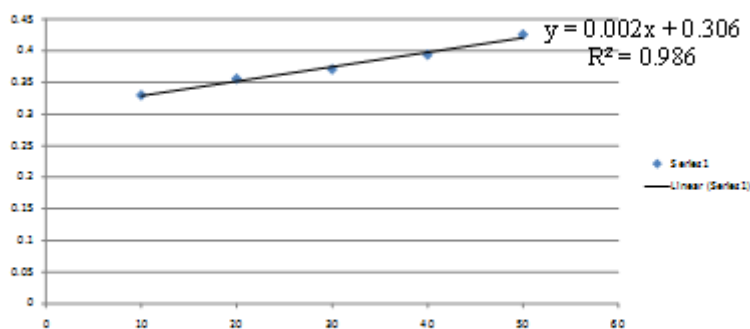
Preliminary phytochemical studies were performed to detect the presence of tannins, flavonoids, anthocyanins, saponins, and alkaloids according to the method described by Kokate, 1994 and Harborne, 1998. The tests were based on the visual observation of color change or formation of a precipitate after the addition of specific reagents. The results are shown in Table 1.

Table 1: Preliminary phytochemical analysis of Various extracts of *S. splendens* Leaves

S.No.	Phyto-constituents	Petroleum ether (60-80)	Chloroform	Methanol
1.	Alkaloids	–	–	++
2.	Anthocyanins	+	+	+++
3.	Carbohydrates	–	–	+
4.	Flavonoids	–	–	+++
5.	Phenols	–	–	++
6.	Glycosides	–	+	++
7.	Reducing sugars	–	–	+
8.	Saponins	–	–	+
9.	Sterols	++	–	+
10.	Terpenoids	–	+	+++
11.	Tannins	–	+	+

Determination of the total Phenolic content:

Total phenol content was measured by Folin-Ciocalteu reagent method [21, 22]. 1mg/ml of dry extract solution was prepared in methanol, to this 0.5 ml of 10% folin-Ciocalteu reagent and 0.5 ml of sodium carbonate and volume made up to 10 ml with distilled water. Solution was incubated for 2hr and absorbance was measured at 765 nm. Concentration was determined from standard graph using gallic acid and result was mentioned as mg equivalent to gallic acid [23, 24]. The total phenolic content was found to be 17 mg of gallic acid equivalent per 1gm of methanolic extract.(Fig 1).

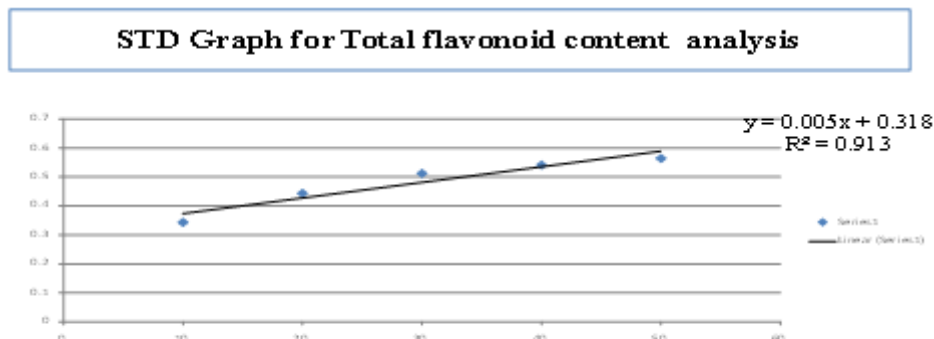
Standard Graph of Gallic acid

▪The total phenolic content was found to be 17 mg of gallic acid equivalent per 1gm of methanolic extract.

Fig 1: Determination of the total Phenolic content

Determination of the total flavonoid content:

0.5 ml of the sample (100 µg/ml) was mixed with 1.5 ml of methanol (75% v/v), 0.1 ml of aluminium chloride (10% w/v), 0.1 ml of potassium acetate (1M) and 2.8 ml of distilled water. The mixture was allowed to stand for 30 minutes in room temperature. The absorbance was measured at 435 nm using spectrophotometer. Quercetin was used as standard and results were expressed as mg/g quercetin equivalent. The total flavonoid content was found to be 117.4mg of Quercetin equivalent per 1gm of methanolic extract[25](Fig 2)



■The total flavonoid content was found to be **117.4mg** of Quercetin equivalent per 1gm of methanolic extract

Fig 2: Determination of the total Flavonoid content

***In vitro* anti oxidant activity of extracts of *S. splendens* Leaves:**

- (a) Free radical scavenging activity using DPPH.
- (b) Hydroxyl radical scavenging activity.

(a) Free radical scavenging activity using DPPH:

The ability of the samples to scavenge the free radicals was estimated by *in vitro* method using a stable nitrogen centered radical viz. DPPH [26]. Extract 0.05 ml dissolved in methanol was added to a methanolic solution of DPPH (100 µM, 2.95 ml) at different concentration (200-1000 µg/ml) and the absorbance was recorded at 517 nm (Perkin Elmer-1700).

$$\text{DPPH scavenging activity (\%)} = \frac{[AC - AS]}{AC} \times 100$$

where AC is the absorbance value of the control and AS is the absorbance value of the added test samples solution.

(b) OH radical scavenging activity mediated 2-deoxy-D-ribose degradation:

The reaction mixture contained ascorbic acid (50 µM), FeCl₃ (20 µM), EDTA (2 µM), H₂O₂ (1.42 mM), deoxyribose (2.8 mM), with different concentrations of samples in a final volume of 1 ml in potassium phosphate buffer (10 mM, pH 7.4). It was incubated at 37°C for 1 h and then 1 ml of 2.8% TCA and 1 ml of 1% TBA were added. The mixture was heated in a boiling water bath for 15 min. It was cooled and absorbance was measured at 532 nm with spectrophotometer (Schimadzu-1700) (Table 2).

Minimum inhibitory concentrations (MIC)

The MIC was done by the method described by Ver-poorte [27]. The extracts were incorporated into Mueller-Hinton broth at concentration ranging from 0.01-10mg/ml. A control tube containing the growth medium and the bacteria was set-up. The mixtures were incubated for 24hs. The minimum inhibitory concentration (MIC) of the extracts was regarded as the lowest concentration of the extract that did not permit any turbidity or growth of the test organisms. (Table 3).

**Invitro anti oxidant activity of the various Fractions of
methanolic extract of leaves of *Salvia splendens***

Test	IC ₅₀ Values (µg/ ml)			Inhibition %	
	Ethyl acetate fraction	N-butanol fraction	Aqueous fraction	Ascorbic acid	Quercetin
DPPH	104.18	>1000	>1000	92.13	-
OH radical	208.78	940.58	>1000	-	83.71

Table 3: Minimum Inhibitory concentration of Methanolic extract of *Salvia splendens* leaves

Microorganisms	MTCC No.	MIC in µg/ml	
		Methanolic Extract of <i>Salvia splendens</i>	Standard
		Gentamycin	Amphotericin B
<i>Staphylococcus aureus</i>	MTCC 9760	200±2.57	1±0.021
<i>Streptococcus pyogenes</i>	MTCC 1925	350±3.12	2±0.018
<i>E. coli</i>	MTCC 1679	400±3.65	1±0.011
<i>Pseudomonas aeruginosa</i>	MTCC 4673	400±3.17	2±0.01
<i>Candida albicans</i>	MTCC 854	350±3.93	NA
<i>Aspergillus niger</i>	MTCC10180	350±3.76	0.5±0.001

MIC, minimum inhibitory concentration; values are given as µg/ml. NA, not applicable. M±S.D, mean of three experiments± standard deviation.

CONCLUSION

Methanol extracts of *S.splendens* leaves exhibited antioxidant and antimicrobial activities. These effects might be due to the unsaturated fatty acids, sterols, triterpenes phenolic acids and flavonoids in the methanol extract. These results showed that *S. splendens* extracts could be considered as natural antioxidant and antimicrobial agents.

Acknowledgements

The authors would like to acknowledge Botanical Survey of India, Kolkata for authentication of plant specimen & Department of Pharmaceutical Sciences & Technology, Birla Institute of Technology, Mesra, Ranchi, India for providing the research facilities to carry out this activity.

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