Research articles

The Effect of Solvents to Extract Phenol and Phenol Related Compounds Contents and Some Biological Screening of Salvia *officinalis*

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Abstract

Saliva officinalis (Lamiaceae) herbal plant collected from Khartoum local market. This study has three aims, to assess the efficacy of three aqueous organic solvents to extract the phenol and phenol derivative metabolites using the maceration method, to assess the antifungal and anticancer of Saliva officinalis aqueous extracts using Yeast – Based bioassay against three mutant yeast strains Saccharomyces cerevisiae, and to assess the antioxidant activity of Saliva officinalis extracts using two methods: DPPH radical scavenging assay, and TLC Autography technique. The results revealed that the 70% methanol has high total phenols (318.35mg GAE/g) and total flavonoids (189.38mg QE/g) while the 70% acetone extract was revealed high total tannin content (218.34 mg TAE/g). The 70% acetone extract revealed activity against the three mutant yeast tested with inhibition zone diameter more than 12 mm at concentration 5mg/ml and 10mg/ml respectively. While the other extracts were exhibited no activity against the three mutant strains tested. All extracts were reflected anti-Candida activity and the maximum IZD was exhibited by the 70%ethanol extract (30mm). All extracts were free-radical inhibitors but 70% methanol extract was more potent (0.068mg/ml) than the other extracts

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Keywords

Saliva officinalis, Polyphenols, Flavonoids, Tannin, Antioxidant Activity, Anticandidal Activity,

Topoisomerase I, Topoisomerase II

I. Introduction

S aliva officinalis show immense potential therapeutic action in traditional medicine in Sudan. Candidal infection and obesity are reflecting on the serious causes of many diseases [(1)].

Phenols and phenol containing compounds having an important physiological action on the human body [(2.3)]. Solvents and extraction methods must be carefully chosen to optimize meatabolits extraction and their biological activity [(4, 5and 6)].

Salvia officinalis is a common sage, evergreen sub-shrub with woody stems, grayish leaves, and blue to purplish flowers native to Southern Europe and the Mediterranean region. Traditionally it was used as herbal tea in the treatment of diarrhea, colic, dyspepsia, constipation, asthma, bronchitis, catarrh, cough, and depression [(7)]. Chemically Saliva officinalis consist of essential oil, Phenolic acids (muscarinic), carnosic acid, diterpenoids, triterpenoids, flavonoids and phenolic glycosides (8,9,10,11and 12).

II. Material and Methods

Plant material

Salvia officinals were collected from local markets during September (2016) and authenticated in Herbarium of Medicinal and Aromatic Plants and Traditional Medicine Research Institute, National Center for research, Khartoum, Sudan.

Extraction

The whole plant ground to a coarse powder. A weight of the coarse powder (100g) was macerated in 1000ml aqueous organic solvent at room temperature (27°C) for 48 hrs. The organic solvents used were 70% ethanol, 70% methanol, 70% acetone, and distilled water. The macerated material was filtrated and concentrated using a rotary evaporator and kept at 4°C till used.

Determination of the Total Contents Total Phenol Content

The total phenolic content of extracts was determined with Folin-Ciocalteu reagent. The extract (10 mg/mL) was mixed with 2.5 mL of Folin-Ciocalteu reagent into test tubes and 2 mL of sodium carbonate solution were added. The tubes were vortexed and incubated at room temperature for 15 min. afterward, absorption was measured at 765 nm. Galic acid was used as standard. The total phenol

values are expressed in terms of gallic acid equivalent (GAE) [13].

Total Flavonoids Content

The total flavonoid content of the extract was determined with quercetin reagent.

0.3ml extract were mixed in a test tube with 0.3ml of sodium nitrite (5%) and incubated for 5min. at room temperature, 0.3ml of aluminum chloride solution (10%) was added and incubated for 5min, then 2ml of sodium hydroxide (1M) was added. Absorbance was measured at 415nm against a reagent blank. The quercetin (10mg/ml) was used as control. The total flavonoid content was expressed in terms of quercetin equivalent (QE) [13].

Total Tannins Content

The total tannins content of the extract was determined using $FeCl_3$ and gelatin test with some modification. One ml of extract (1mg/ml) was transferred to vials, 1ml of 1% $K_3Fe(CN)_6$ and 1ml of $FeCl_3$ were added, and the volume was made up to 10ml with distilled water. After 5min absorbance was measured at 510nm against a reagent blank. The total tannin content was expressed in terms of tannic acid equivalent (TAE)[13].

Anticandidal Activity

Cup-Plate Agar Diffusion Method

A concentrated dry crude extract was prepared at concentration 0.1g/ ml up to 0.05g/ml with sterile distilled water.

A loop full Candida albicans was thoroughly mixed with 100 ml sterile nutrient agar and poured into sterile Petri-dishes (20ml/ dish). The prepared plates were left at room temperature to set. In each prepared plat four wells, (100 µl capacity and 10 mm in diameter using a sterile cork borer No.4) were done. The cups were filled with 100 µl of tested crude extracts using an automatic microliter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 h. Two replicates were carried out for each extract. The activity was measured by measuring the inhibition zone diameter [14].

Yeast - Based Bioassay for DNA – Damaging

Three mutant strains of Saccharomyces cerevisiae yeast were used: wild strain, strain with deficient in Topoisomerase I enzyme, and strain with deficient in Topoisomerase II enzyme. Sex wells of 100µl capacity were done in an inoculated agar plate with yeast strain. The well was filled with the crude extract and incubated at 37ºC for 48hrs. The activity was measured by measuring the inhibition zone diameter. The concentration of extract resulted in 12 mm inhibition zone diameter was accepted as inhibition concentration [14].

In-Vitro Determination of Antioxidant Capacity

I. DPPH radical scavenging assay Radical scavenging activity of extracts against stable 2,2-diphenyl-2picrylhydrazyl hydrate (DPPH) was determined spectrophotometrically. The extracts were prepared by dissolving in methanol. The solution of DPPH in methanol (6 × 10-5 M) was prepared daily before the UV measurements, and 3 ml of the solution was mixed with 77 μL of extract solution. The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured at 515 nm on a spectrophotometer. BHT (50 μg/mL) was used as a reference compound. The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula:

% inhibition = [(AC – AS) / AC] × 100, Where AC is the absorption of a blank sample (t = 0 min) and AS is the absorption of tested extract solution (t = 15 min).

II. TLC Autography technique The extract resolved in the solvent is spotted on the silica-gel 60F 254 plates and develop the chromatogram inadequate solvent systems. The developed dry plates sprayed with a methanolic solution of DPPH (2mg/ml). Thus, antioxidants appear as yellow bands on a light purple background [15].

III. Results Yield percent

The maximum yield was determined by water extract followed by aqueous methanol, while the aqueous acetone was reflected in the minimum yield percent table (1). All extracts having a pleasant aromatic smell and brown color with different in the intensity.

Table 1: Yield Percent of Saliva officinalis Whole Extracts

Extractive Solvent	Yield %	Organoleptic Properties of Extract
70% Methanol	10.48	Deep brown, pleasant smell, soluble in water and alcohol
70% Ethanol	8.67	Brown, pleasant smell, soluble in water and alcohol
70% Acetone	7.89	Yellow wish brown, pleasant smell, soluble in water and alcohol
Aqueous	12.54	Dark brown, pleasant smell, soluble in water and alcohol

Phytochemical Screening

The preliminary phytochemical screening of the different extracts of Salvia officinalis showed the presence of all metabolites tested except saponins (Table: 2).

Table 2: Phytoconstituents of Saliva officinalis Whole Extracts

Metabolite	Plant Extract			
	Methano 70%	Ethanol 70%	Acetone 70%	Aqueous
steroid	+	+	+	+
Phytosterols	+	+	+	+
Terpenoids	+	+	+	+
Alkaloids	+	+	+	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Saponins	-	-	-	-

Phenol and Phenol Derivatives Total Contents of Saliva officinalis Saliva officinals70% ethanol extract revealed the highest flavonoid and tannin contents while 70% acetone extract was revealed high total tannin content (Table: 3)

Table 3: Total Phenolic, Total Flavonoid, and Total Tannins of Saliva officinals

Plant Extract	Total contents		
	Phenols (mgGAE/g)	Flavonoids (mgQE/g)	Tannins (mgTAE/g)
Methanol (70%)	318.35	189.38	155.4
Ethanol (70%)	304.54	115.84	55.74
Acetone (70%)	249.54	58.45	218.34
Aqueous	114.14	83.61	88.24

In-Vitro Antioxidant Capacity of Saliva officinalis

The antioxidant activity of Salvia officinalis extracts was assessed using two methods: DPPH radical scavenging assay, and TLC Autography technique. DPPH radical scavenging assay was revealed that all extracts were free radical inhibitors but 70% methanol extract was more potent (0.068mg/ml) than the other extracts (Table: 4). This result was supported by the TLC Autography technique in which all extracts exhibited antioxidants as yellow bands on a light purple background (Photo: 1).

Table 4: Antioxidant Activity of Saliva officinalis Extracts using DPPH

Plant Extract	IC50 (mg/ml) ± SD
Methanol 70%	0.068 ±2.31
Ethanol 70%	0.800 ±0.15
Acetone 70%	0.221 ±3.14
Aqueous	0.113 ±0.21

Anti-Candida Activity

Saliva officinalis all extracts were reflected anti-Candida activity by diffusion method. The 70% acetone revealed the maximum IZD at concentration 10 mg/ml against

Candida albican standard strain tested (46mm) and Candida albican isolated strain tested (37mg/ml). The activity is concentration-dependent (Table: 5).

Table 5: Activity of Saliva officinalis extracts Against Candida albican Standard and Isolated strains at Different Concentrations

PlantExtracts	Inhibition Zone Diameter (mm)										Mycohealoralgel (100 mg/ml)	
	Plant Extract Concentration (mg/ml)											
	0.5		1.0		2.5		5.0		10			
	Candida albican strains											
	std	lso	std	lso	std	std	lso	std	std	lso	std	lso
Methanol	17	15	16	14	16	15	20	22	28	29	30	21
Ethanol	12	12	12	15	12	11	22	20	38	30		
Acetone	20	19	29	22	29	24	34	30	46	37		
Aqueou	16	15	20	12	20	19	20	20	29	29		

Key: St=Standard strain **Is=** Isolated strain

Yeast - Based Bioassay for DNA – Damaging

The 70% acetone extract was revealed activity against the three mutant yeast tested with inhibition zone diameter 19, 20, and 22mm at concentration 10mg/ml. While the other extracts were exhibited no activity against all strains tested (Table: 6).

Table 6: Activity of Saliva officinalis extracts against three mutant strains of Saccharomyces cerevisiae at Different concentrations

PlantExtracts	Inhibition Zone Diameter (mm)															
	Plant Extract Concentration (mg/ml)															
	0.5			1.0			2.5			5.0			10			
	Saccharomyces cerevisiae															
	W	TI	TII	W	TI	TII	W	TI	TII	W	TI	TII	W	TI	TII
Methanol70%	8	11	9	7	8	8	9	4	6	8	7	9	9	9	9
Ethanol70%	5	8	7	9	6	9	8	8	9	7	9	9	8	10	8
Acetone70%	6	8	7	8	9	8	7	10	9	11	10	11	22	19	20
Aqueous	0	3	2	5	6	3	8	5	6	4	4	6	0	8	9

Key: **W**= wild type of yeast, **TI**= type of yeast has deficient in Topoisomerase I enzyme but has Topoisomerase II, **TII**= type of yeast has deficient in Topoisomerase II enzyme but has Topoisomerase I.

III. Discussion

Saliva officinalis is represented as a potent medicinal plant, traditionally plays an important role in the treatment of fungal infection especially oral cavity infection this may be due to the presence of phenolic compounds. Phenolic compounds are one of the major groups of metabolites in the plant. They represent diversity in the chemical properties and accordingly they classified into water-insoluble and water-soluble compounds [6]. The yields of extract depend on many factors one of them is the solvent with varying polarity [13]. The yield of water is the higher one followed by 70% methanol extract yield than 70% ethanol followed by 70% acetone. This result was supported by different scientific reports [11, 17, 18]. That means the perfect solvent to get the polar metabolites of this plant is the water than the others. Compounds other than phenolics may have been extracted and contribute to higher yield. This may be attributable to the higher solubility of proteins and carbohydrates in water and methanol than in ethanol and acetone [14].

The maximum total content of both phenols and flavonoids was recorded in 70% methanol extract (318.38 mg GAE/100G and 189.38mg QE/100g) respectively, while the aqueous acetone extract was exhibited a high tannin total content (218.34 TAE/100g). Based on this result, the best extracting solvent of the phenolic compounds and flavonoids was 70% methanol, while 70%acetone is the best extracting solvent of tannin this result agrees with [16].

All extracts were exhibited antioxidant activity. The result revealed by DPPH radical scavenging assay was supported by the TLC Autography technique. The 70% methanol extract was showed the highest antioxidant activity, which is correlated to the total flavonoid content (10, 8 15) and general agreement with (13, 14,15, 17).

All extracts were inhibited the activity of candida albicans both isolated strain and standard strain at concentration 5mg/ml and 10mg/ml. The 70% acetone extract was reflected as a maximum inhibition activity at all concentrations tested. This result is explained by the content of tannin in the 70% aqueous ethanol.

Saliva officinalis 70% acetone extract (0.5g/ml) revealed activity against the three mutant Saccharomyces cerevisiae tested with inhibition zone diameter 19, 20, and 22mm at concentration 5mg/ml and 10mg/ml. That means Saliva officinalis polar metabolites have antifungal agents and anticancer agents [17, 18].

From the results in this research some trends were indicating the impact of extractive solvent on the total contents and the yield of phenol and phenol derivatives metabolites. On the other hand, the correlation between the type of natural compound and its antifungal, antioxidant and anticancer activity was found.

IV. Conclusion

The choice of the solvent to extract any metabolites from its biomass is affect not only on the yield% of the metabolites but also on the therapeutic activity. The plant Saliva officinalis have a high oxidative power so it is so promising and having a high activity against candida albicans which supports its traditional use.

The results were reflected a clearly correlations between types of seeds metabolites extracted and their antifungal activity, so it could be necessary to look deeper into subgroups of compounds, instead of grouping the metabolites into biosynthetic groups.

V. Acknowledgment

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VI. References

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