

Methanolic Extracts Antioxidant and Antimicrobial Activities from Five Varieties of Common Beans (*Phaseolus vulgaris* L.)

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Abstract

The aim of this study was the antioxidant determination by the DPPH method and the antimicrobial determination by the agar diffusion susceptibility technique of methanolic extracts at 50, 80 and 100 % (v/v) from bean flours (varieties: Zacatecas, Jamapa and plus black; speckled Saltillo and sulphur yellow). The phytochemical screening of the seeds showed that the extracts contained flavonoids, tannins, and saponins, which could be responsible for the antimicrobial and antioxidant activities. Higher antioxidant activity (90 %) was observed in pure methanol extracts (Duncan, $p \leq 0.05$); also it was observed that varieties with flavonoids conserved their activity in methanol:water extracts. Antimicrobial activity was observed in the methanolic extracts (50:50) principally against *Salmonella typhi* (MIC = 0.125 mg/mL with Jamapa black bean). Varieties with saponins and flavonoids presented both activities; it means that dark pigmented coats possess higher potential as health enhancer than those with light coloured seed coats.

Key words: *Phaseolus vulgaris* L., phytochemical profile, antioxidant, antimicrobial, methanolic extract, biological activity.

1. Introduction

Recently, human are interested in finding active compounds, which can improve the human health; principally if such compounds are from natural sources. Nowadays, there are unexploited sources like plants. Some foods from vegetable origin could contain several active compounds, which are not widely studied [1]. Plants synthesize some primary metabolites like nucleic acids, proteins, lipids and carbohydrates which are essentials for their growth and development; and some secondary metabolites usually derived from the

primary metabolism like flavonoids, alkaloids, essential oils, glycosides, saponins and tannins, which contribute in several biological activities [2-3]. The saponins and flavonoids found in beans have been widely studied due to their protective role against cardiovascular diseases and cancer; saponins have been demonstrated to be valuable antitumor promoters in carcinogenesis due to their antioxidant effect, direct and selective cytotoxic effect against cancer cells and regulation of cell proliferation, especially if combined with certain flavonoids [4]. Antioxidant compounds could delay the cellular ageing natural process. During the oxidation process and through the metabolism some free radicals are release, such radicals could saturate protection enzymes like the dismutase, catalase and peroxidase; resulting in cellular death and damage due to the membrane lipids oxidation, cellular proteins, DNA and related enzymes. Oxidative stress is an important factor in several diseases age related like diabetes, cancer and atherosclerosis. Recent studies suggest that some foods could inhibit free radicals and act as antioxidants due to their composition [5-6]. In other hand, antimicrobial compounds have an important role in food and health, although mainly are from synthetic origin. In the food industry, numerous researches had been done to implement natural compounds (like essential oils, bacteriocins and plant extracts) with the purpose to replace the consumption of synthetic compounds avoiding the consequences in long term of these kinds of products [7]. Previous reports suggest the secondary metabolites extraction from vegetable sources using dissolvent and some *in vitro* activities such as: anti-inflammatory, antimutagenic, antioxidant, antimicrobial and antifungal have been proved [8-12]. Common bean (*Phaseolus vulgaris* L.) is a habitual food leguminous in the Mexicans diet, these seeds contribute with around 22 g / 100 g of vegetable protein [6]; and they contain other compounds associated with several biological activities [13].

The aim of this work was to determine the antioxidant and antimicrobial capacities of methanolic extracts obtained from five bean (*Phaseolus vulgaris* L.) varieties.

2. Materials and methods

2.1 Preliminary bean flour treatment

Seeds used were plus black bean (PB) FRI-064-2109993, sulphur yellow bean (SY) FRI-001-220995 and speckled Saltillo bean (SS) 1424-FRI-026-120901/C, donated by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP, Mexico); Zacatecas black bean (ZB) and Jamapa black bean (JB) were purchased in a local market. Manually impurities were retired from the seeds. Seeds were grinded in a manual mill and defatted by hexane (1:5 w/v), with constant stirred 8 h at 4 °C, finally flour was sieved until mesh number 80 [14].

2.2 Methanolic extraction

In this test three methanol:water mixtures were used 50, 80 y 100 % (v/v). Beans flours at 10 % (w/v) were dispersed in the dissolvent and stirred 24 h at room temperature. Samples were filtrated through filter paper (Whatman™ No. 40) and concentrated in a rotavapor R-205 (Büchi, USA). All extracts were weighed and stored at 4 °C until use [15].

2.3 Phytochemical profile of bean flour methanolic extracts

For the initial determination of compounds (secondary metabolites) presented in the methanolic extracts of five bean varieties a chemical identification probes were done. In all samples the presence of flavonoids (Shinoda and NaOH reactions), reducing sugars, tannins (gelatin, ferric chloride and potassium ferrocyanide reactions), and saponins (Lieberman-Bouchard and Rosenthaler reactions) were detected by a phytochemical profile test following an established protocol [16].

2.4 Antioxidant activity determination of bean methanolic extracts

The free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) capture was determined according to Thomas *et al.* [17]. 2.9 mL of DPPH+ 0.1 mM

methanolic solution were mixed with 0.1 mL of the samples (1 mg/mL). Absorbance (A) was red at 30 and 60 min of reaction time at 515 nm in a Lambda XLS spectrophotometer (Perkin Elmer, USA) using methanol as blank. Every measurement was repeated three times. The free radical DPPH⁺ scavenging (reduction) activity was calculated from the equation: Antioxidant activity [%] = [(A-A_x)/A]*100, where A is the absorbance of DPPH⁺ solution, and A_x is the absorbance of a DPPH⁺ solution with a tested extract solution.

2.5 Antimicrobial activity of bean methanolic extracts

Strains used in this test were: *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Escherichia coli*, *Klebsiella pneumoniae*, *K. rhinoscleromatis*, *Salmonella typhi*, *Shigella dysenteriae* and *Enterobacter agglomerans*. The susceptibility probe was carried out according to Bauer *et al.* [18] method. Antibiotic chloramphenicol (3 mg/mL) was used as positive control and water as negative control. The minimum inhibitory concentration (MIC) assay was carried out by the broth dilution method following the Langfield *et al.* [19] method in samples with previous activity using 0.1 % (w/v) tetrazolium chloride (2,3,5-triphenyl-2H-tetrazolium chloride) as indicator of cellular life.

2.6 Statistical analysis

An analysis of variance (ANOVA) was conducted, and a Duncan's test (Duncan, p≤0.05) was used to find statistically significant differences between samples. Both analyses were carried out by SAS 9.0 for Windows (SAS Institute Inc., USA).

3. Results and discussion

3.1 Phytochemical profile of bean flour methanolic extracts

Before carried out the antioxidant and antimicrobial assay, the five bean flours were characterized with a preliminary test in order to know which secondary metabolites were in them, results are presented in Table 1. In the samples, flavonoids (flavones, xanthones, flavonols and anthocyanins), tannins (catechol and galic acid derivates) and saponins triptenoids were

presented. It has been reported that in *P. vulgaris* secondary metabolites like phenolic compounds are mainly located in the seed coat [20]. Also, it was observed that the methanol concentration in the extraction process has influence on the metabolites extracted. Guajardo-Flores *et al.* [4] reported the presence of saponins and flavonoids in black bean extracts, which agree with the results obtained in this work. The flavonoids and saponins groups were observed principally in black beans extracts; authors had reported a biological activity (antimicrobial, antioxidant, antitumoral, hipogluceamiant, etc.) with plant extracts and attributed such activity to these specific compounds [21]. Moreover, Al-Temimi and Choudhary [23] compared diverse pure solvents and observed the highest extractions were with methanol, ethanol and water; this result may support the fact that these beans extractions had high levels of activity. Amarowicz *et al.* [3] reported the presence of tannins in the acetonic extracts from *P. vulgaris* and demonstrated antibacterial activity. Moreover, the amount and composition of flavonol glycosides, condensed tannins and anthocyanins determine the seed colour [20]; dark pigmented coats such as black beans possess higher contents of phenolic than those with light coloured seed coats, this behaviour was observed in our samples.

3.2 Antioxidant activity determination of bean methanolic extracts

DPPH+ free radical inhibition by the beans methanolic extracts was determined (Figure 1) and was observed that samples extracted with 100 % methanol had

antioxidant activity superior to 90 %. When the methanol concentration was decreased to 80 %, the antioxidant activity also decreased significantly (Duncan, $p \leq 0.05$). In bean varieties PB, BZ and SS the presence of flavonoids and saponins were observed and they conserved antioxidant activity up to 80 %, probably due to these metabolites. In 50 % methanol samples the antioxidant activity decreased significantly (Duncan, $p \leq 0.05$) but varieties PB and ZB conserved activity up to 70 %, perhaps because the presence of flavonoids, saponins and tannins. Varieties PB and ZB beans had the highest activity compared to the others (Duncan, $p \leq 0.05$). Vásquez *et al.* [21] linked the antioxidant activity to total phenols contain and reported that the higher presence of phenols in the sample increases the antioxidant activity, probably black bean varieties used in this work have an important phenols contain. Rocha-Guzmán *et al.* [20] reported less antioxidant activity (35.15 %) with higher 50 % black bean methanolic extract concentration (5 mg/mL) and lower in two groups of less pigmented seeds, such behaviour is similar to the results obtained in this work. Moreover, Al-Temimi and Choudhary [23] compared eight techniques to determine antioxidant activity and reported DPPH method as the most simple and easy in light of its low costly; these make possible its application in extracts antioxidant capacity determination. Although, the best vegetable extractions were reported in methanol, ethanol and water solutions [23], some authors suggest the use of chloroform and 1-butanol mixtures to increase antioxidant activity (up to 80 % with less sample quantity, 0.250 mg/mL) [24]. Opposite to this work,

Table 1. Phytochemical screening of five bean varieties methanolic extracts

Metabolite	Bean variety extract Methanol conc. (%)	PB			ZB			JB			SS			SY		
		100	80	50	100	80	50	100	80	50	100	80	50	100	80	50
Flavonoids	Flavones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Flavonols	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Flavanones	+	+	+	+	+	+	-	+	+	-	-	-	+	+	+
	Xantones and flavones	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
	Flavonols	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	Anthocyanins	-	-	-	+	+	+	-	-	+	-	-	-	-	-	-
Reducing sugars	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Tannins	Tannins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Galic acid	-	+	+	-	-	+	-	-	-	-	-	-	-	-	
	Catechol	+	-	-	-	-	-	+	+	-	+	+	+	+	+	
Foam formation and stability	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Saponins	Steroidals	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Triterpenoids	+	+	+	-	-	-	-	-	-	-	-	-	-	-	
	Triterpenoids	+	+	+	+	+	+	+	+	+	-	-	+	-	-	

Qualitative phytochemical analysis of five beans varieties (PB = plus black, ZB = Zacatecas black, JB = Jamapa bean, SS = speckled Saitillo, and SY = sulphur yellow)

Anwar *et al.* [25] reported no significant difference between *Brassica oleracea* L. extracts with several methanol:water mixtures and less reducing power with higher extract concentration (10 mg/mL).

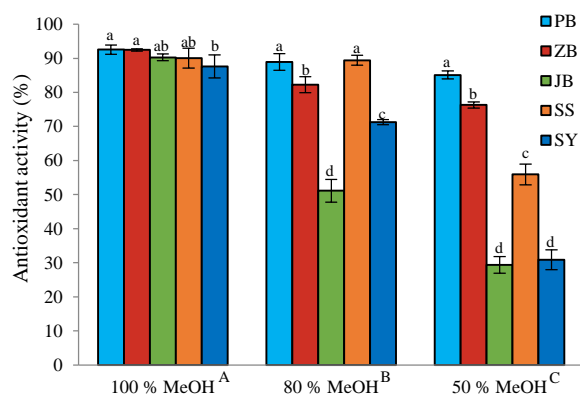


Figure 1. Antioxidant activity presented by the methanolic extracts from five beans varieties (PB = plus black, ZB = Zacatecas black, JB = Jamapa bean, SS = speckled Saltillo, and SY = sulphur yellow). Data points with the same letter are not significantly different (Duncan, $p \leq 0.05$).

3.3 Antimicrobial activity of bean methanolic extracts

No clear trends indicating one variety to be more or less active against the entire range of bacteria tested was noted, although in the disk diffusion susceptibility test with the five beans varieties was positive against *S. typhi*. *S. typhi* presented susceptibility with the 50 % methanolic extracts, *S. aureus* with 100 % PB, and *B. cereus* with 50 % PB, 50 and 100 % SS methanolic extracts, this antimicrobial activity was linked to the secondary metabolites extracted (flavonoids and saponins). Ajayi and Ojelere [1] found bacterial resistance against *B. subtilis* followed by *S. aureus* in *Megaphrynium macrostachyum* seeds extracts and observed that antimicrobial activity increases in higher concentrations of the extracts. The minimum inhibitory concentrations determined for *S. typhi* with the 50 % beans methanolic extracts were: PB 1 mg/mL, ZB 0.50 mg/mL, JB 0.125 mg/mL, SY and SS higher to 1 mg/mL. These results showed that varieties with dark pigmentation have superior antimicrobial activity than other varieties. *S. aureus* with the 100 % PB extract had a MIC of 0.125 mg/mL, and *B. cereus* of 0.25 mg/mL with 50 % PB extract, 0.50 mg/mL with 50 % SS extract and 0.125 mg/mL with 100 % SS extract. Amarowicz *et al.* [3] reported MIC for *P. vulgaris* (red bean variety) acetonetic extracts of 0.125 mg/mL against *L. monocytogenes*, and 0.250 mg/mL against *S. aureus*

and *E. coli*; these results are similar to ones obtained in this work; and also they attributed the antibacterial activity to tannins contained in the samples. Although a MIC of 0.031 mg/mL against *S. aureus* had been reported with other plant extract [19]. Some advantages over the agar disk-diffusion method include the use of small quantities of extract, and the ability to distinguish between bacteriostatic and bactericidal effects besides the MIC determination. Also, the use of a colorimetric indicator eliminates the ambiguity associated with visual comparison [19].

4. Applications

The use of antioxidant compounds presented in bean flours extracts could delay the cellular ageing process avoiding the cellular death and damage. In addition, antimicrobial compounds, which have an important role in food and health, could be obtained from this kind of leguminous in order to improve food preservation or enhancer human health. The efficacy of these beans methanolic extracts as antimicrobials and antioxidants presented a new perspective to all fields that require preservatives and antioxidants of plant origin, mainly if are unexploited.

5. Conclusions

Phenolic compounds were found principally in the beans methanolic extracts such as flavonoids, tannins and saponins. The maximum antioxidant activity was observed in the extracts containing 100 % methanol (up to 90%), then 80 % (up to 80 %) and finally 50 % (from 30 to 70 %). The maximum antimicrobial activity observed was against *S. typhi* when secondary metabolites extraction was with 50 % methanol. Varieties with higher pigmentation presented both activities probably due to the presence of phenolic compounds in their coloured seed coats.

6. References

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