



THE ANTI-AFLATOXIGENIC EFFICACY OF *CYNARA CARDUNCULUS* L. IN SESAME SEEDS (*SESAMUM INDICUM*)



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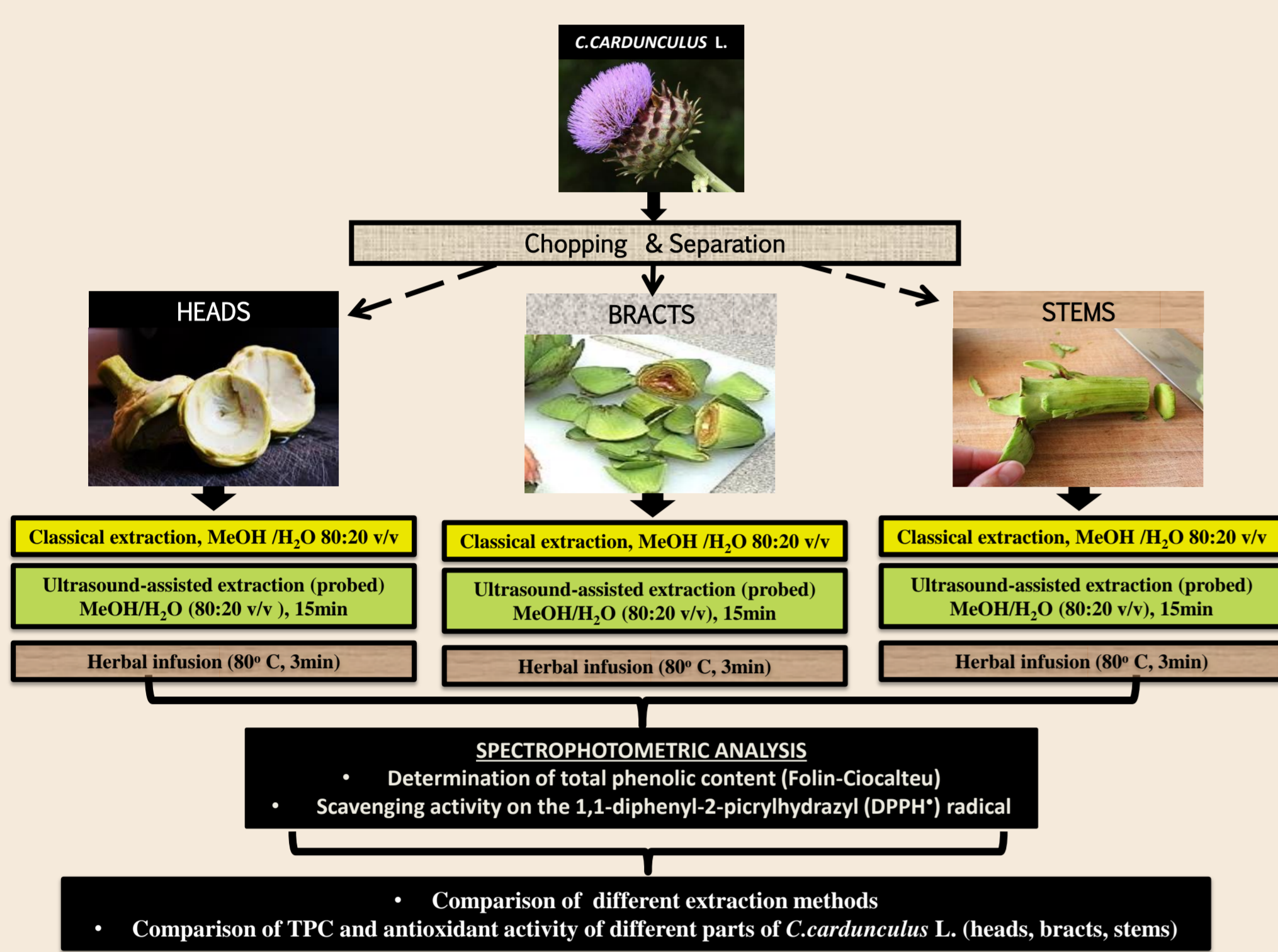
INTRODUCTION

Aflatoxins are a group of secondary metabolites produced by the species: *A.flavus*, *A.parasiticus* and the rare *A.nomius*. Among these, aflatoxin B₁ (AFB₁) is the most naturally occurring compound of toxigenic isolates of *Aspergillus* species and the most dangerous contaminant of foods and feeds due to carcinogenic and mutagenic activity. Sesame seeds (*Sesamum indicum*) are sensitive to AF-producing fungal invasion, because of their oil content, and may therefore be contaminated with AFs and particularly AFB₁. *Cynara cardunculus* L. (*Asteraceae*), commonly named "cardoon" or "wild artichoke", is a Mediterranean species. This multipurpose plant is used worldwide for its edible parts or for its therapeutic properties. However, data related to the antioxidant and anti-aflatoxigenic activities of this species are scarce.

THE AIM OF THIS STUDY WAS TO INVESTIGATE THE ANTIOXIDANT ACTIVITY OF *C. CARDUNCULUS* L. ON AFB₁ PRODUCTION BY *A.PARASITICUS* IN YEAST EXTRACT SUCROSE (YES) & IN SESAME SEEDS.

EXPERIMENTAL DESIGN

1. ANTIOXIDANT ACTIVITY OF *C.CARDUNCULUS* L.



2. EFFECT OF *C.CARDUNCULUS* L. EXTRACT WITH THE HIGHEST ANTIOXIDANT ACTIVITY ON *A.PARASITICUS* MYCELIAL GROWTH

The extract of *C.cardunculus* L. with the highest antioxidant activity, was studied for its effect in *A.parasiticus* mycelial growth in *Aspergillus Flavus Parasiticus* Agar (AFPA).

3. DEVELOPMENT AND VALIDATION OF A METHOD FOR AFB₁ DETERMINATION IN SESAME SEEDS

The analytical protocol for the determination of AFB₁ in sesame seeds was in-house characterized.

4. EFFECT OF *C.CARDUNCULUS* L. EXTRACT WITH THE HIGHEST ANTIOXIDANT ACTIVITY ON AFB₁ PRODUCTION BY *A.PARASITICUS*

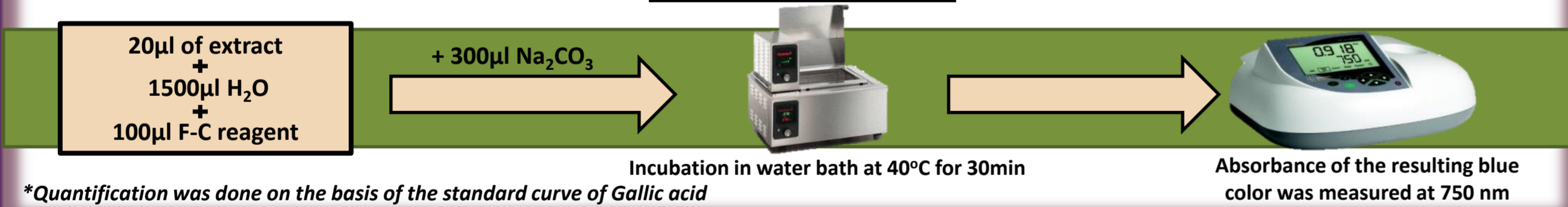
The extract of *C.cardunculus* L. with the highest antioxidant activity, was studied for its effect on AFB₁ production by *A.parasiticus*, in Yeast Extract Sucrose medium & in sesame seeds (*S.indicum*).

GROUPS OF SAMPLES EXAMINED

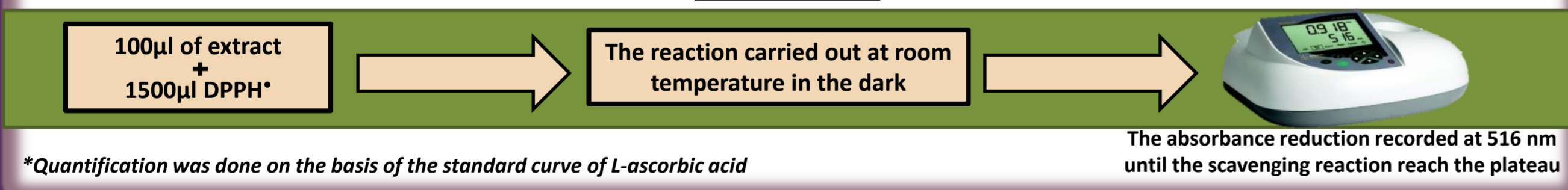
- a₁. YES medium inoculated with *A.parasiticus* (control)
- a₂. YES medium inoculated with *A.parasiticus* and addition of *C.cardunculus* L. extract
- b₁. Sesame samples non inoculated
- b₂. Sesame samples inoculated with *A.parasiticus* (control)
- b₃. Sesame samples inoculated with *A.parasiticus* and addition of *C.cardunculus* L. extract

MATERIALS AND METHODS

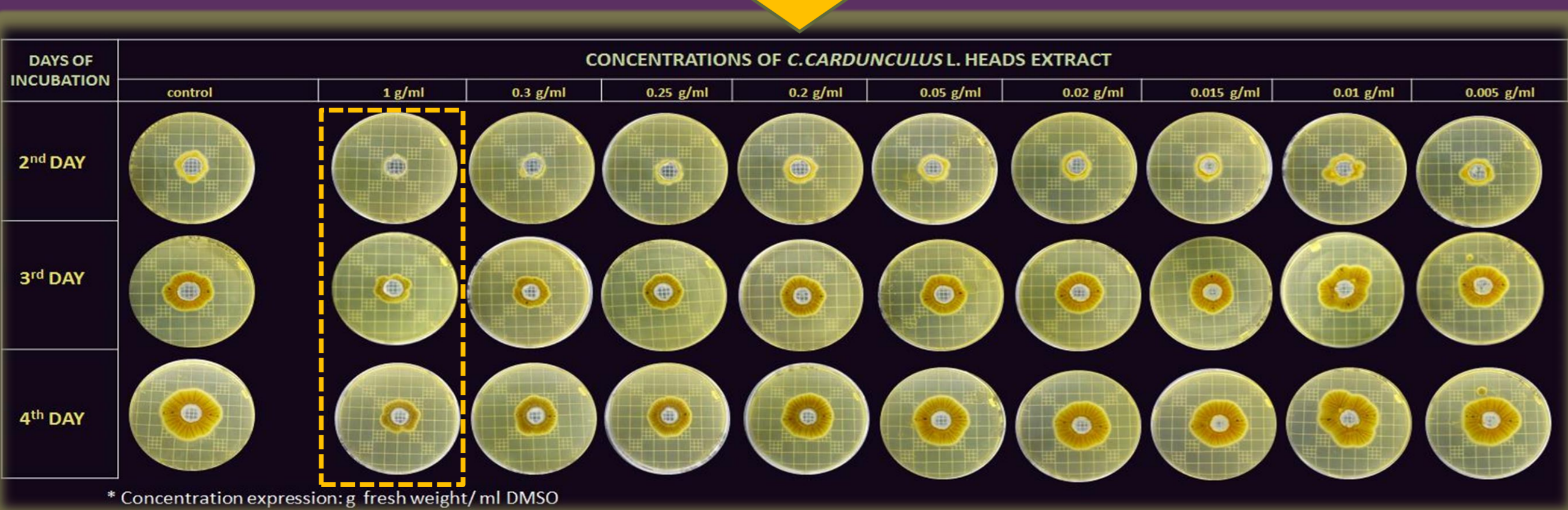
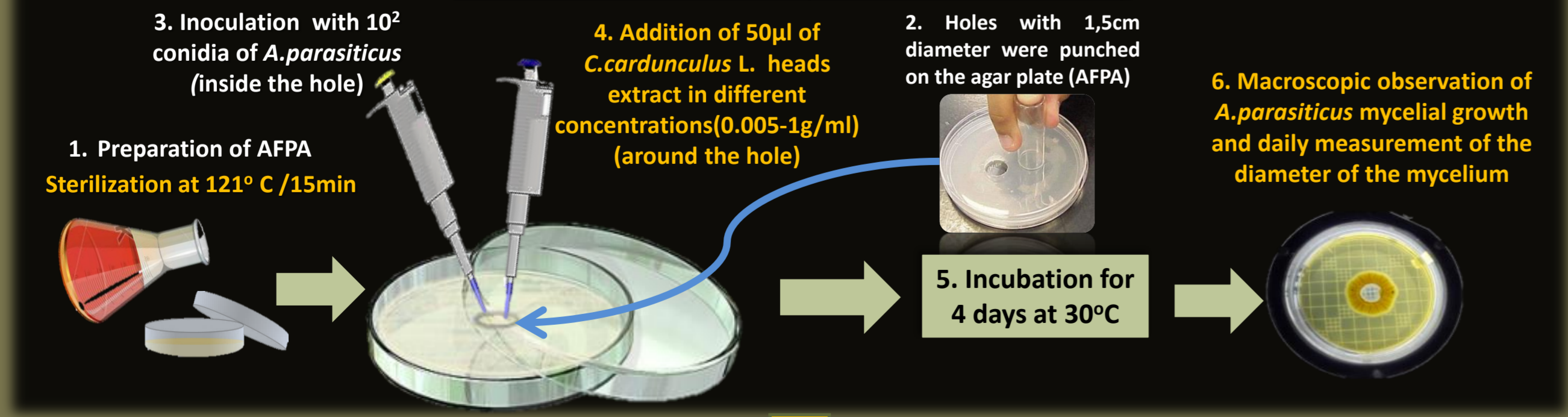
Folin-Ciocalteu method



DPPH method

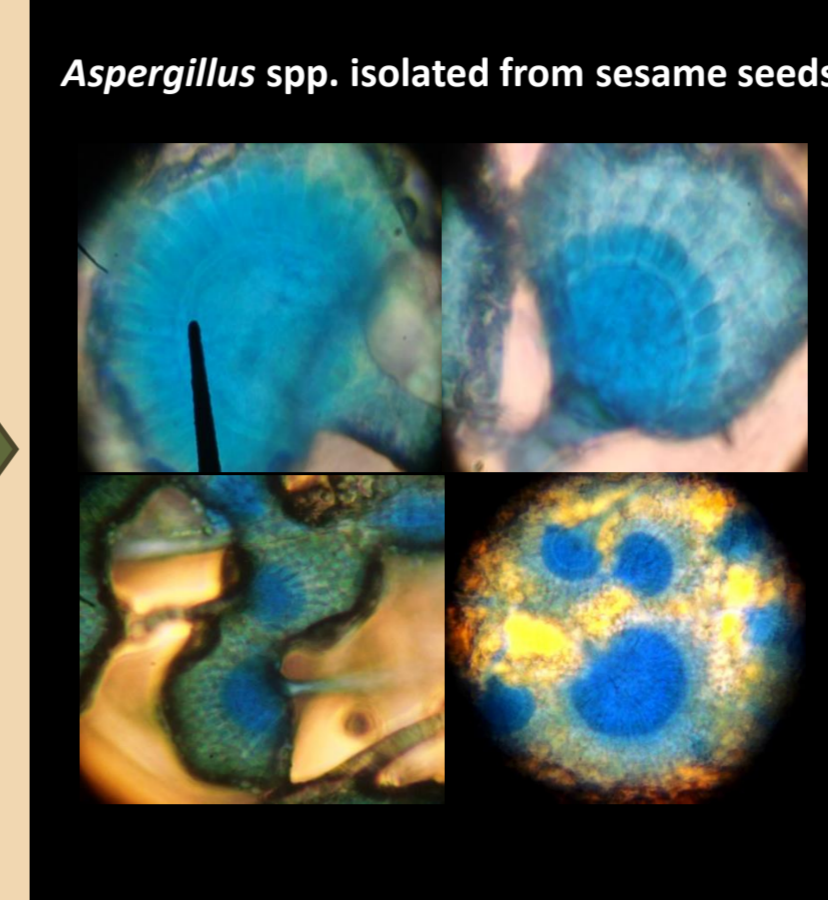


ANTIFUNGAL ACTIVITY (PRELIMINARY STUDY)



EVALUATION OF FUNGAL FLORA IN SESAME SEEDS

Sesame samples transferred into Petri dishes with AFPA
Incubation at 30°C for 5-7 days
Identification of *Aspergillus* colonies according to morphological and microscopic characteristics



SESAME SEEDS TREATMENT BEFORE INOCULATION

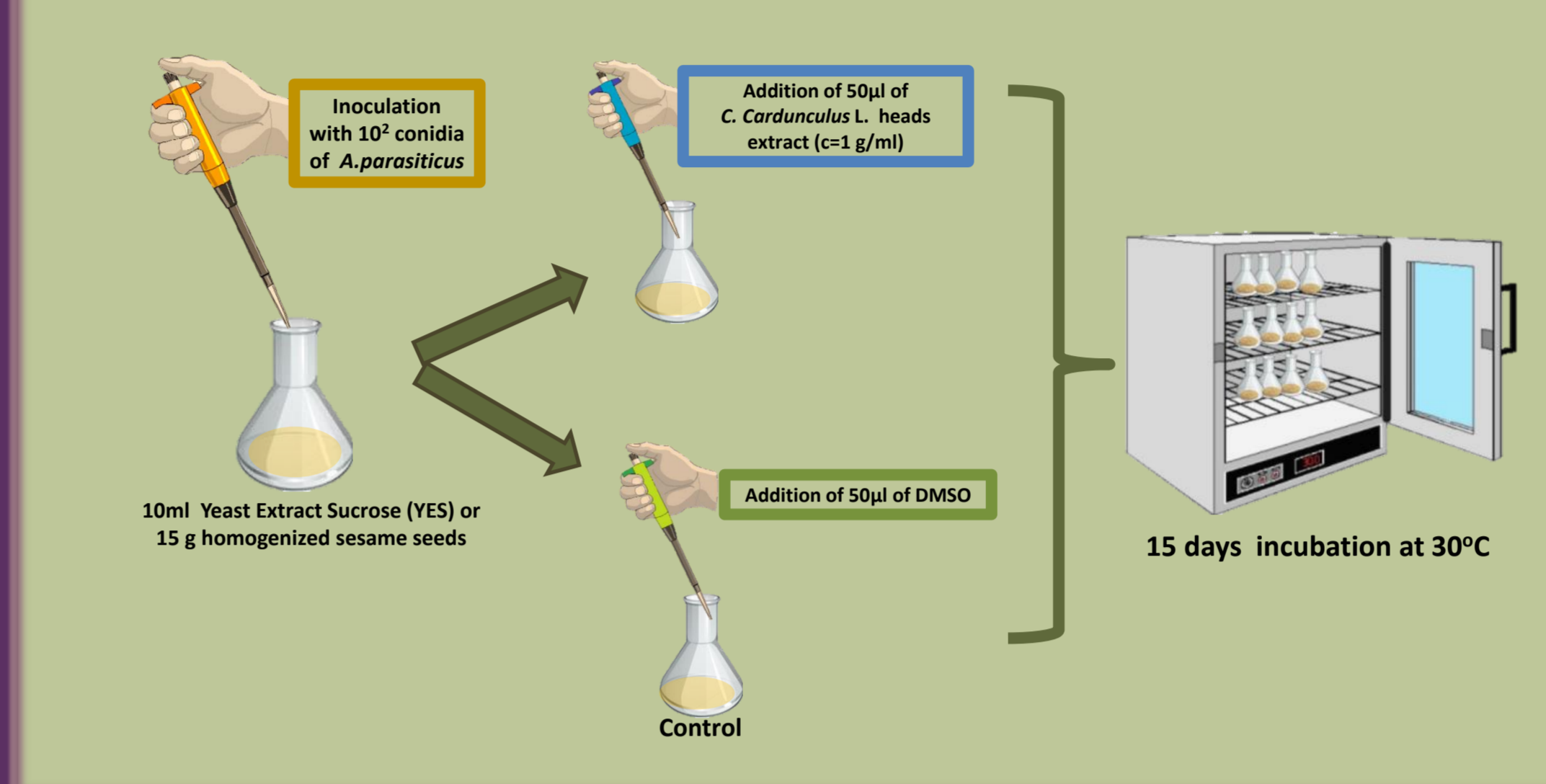
Washed with H₂O
Dry with filter paper
Homogenized (paste)
Heat treatment at 110°C for 2 min

AFB₁ DETERMINATION

Flasks with YES mixed with 30 mL MeOH & flasks with sesame seeds mixed with 30 mL of MeOH:H₂O (80:20 v/v)
10 min extraction
Filtration
1mL Aliquot + 10 mL H₂O (twice)
Immunoaffinity purification
Extraction + 2 mL CH₃CN
Derivatization to AFB_{2a} + 200 µL C₆H₁₄, 200 µL CF₃COOH 40°C/10 min
HPLC

HPLC CONDITIONS
Mobile phase: H₂O:CH₃CN:MeOH 20:4:3v/v/v
Detection: Fluorescence
Ex: 365 nm
Em: 425 nm
Injection volume: 40µL

INOCULATION OF YES MEDIUM & SESAME SEEDS SAMPLES



RESULTS

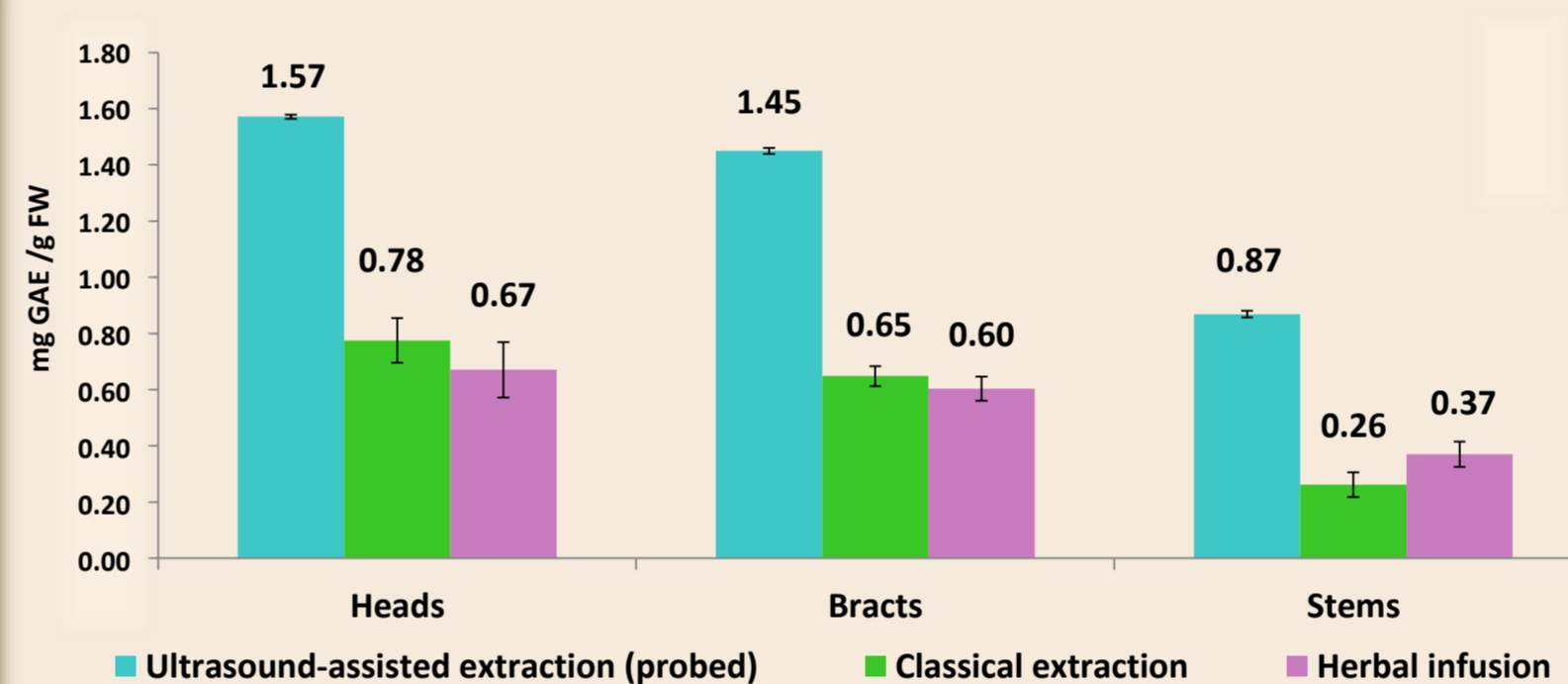


Figure 1: Total phenolic content of *C.cardunculus* L. extracts (heads, bracts, stems) from different extraction methods

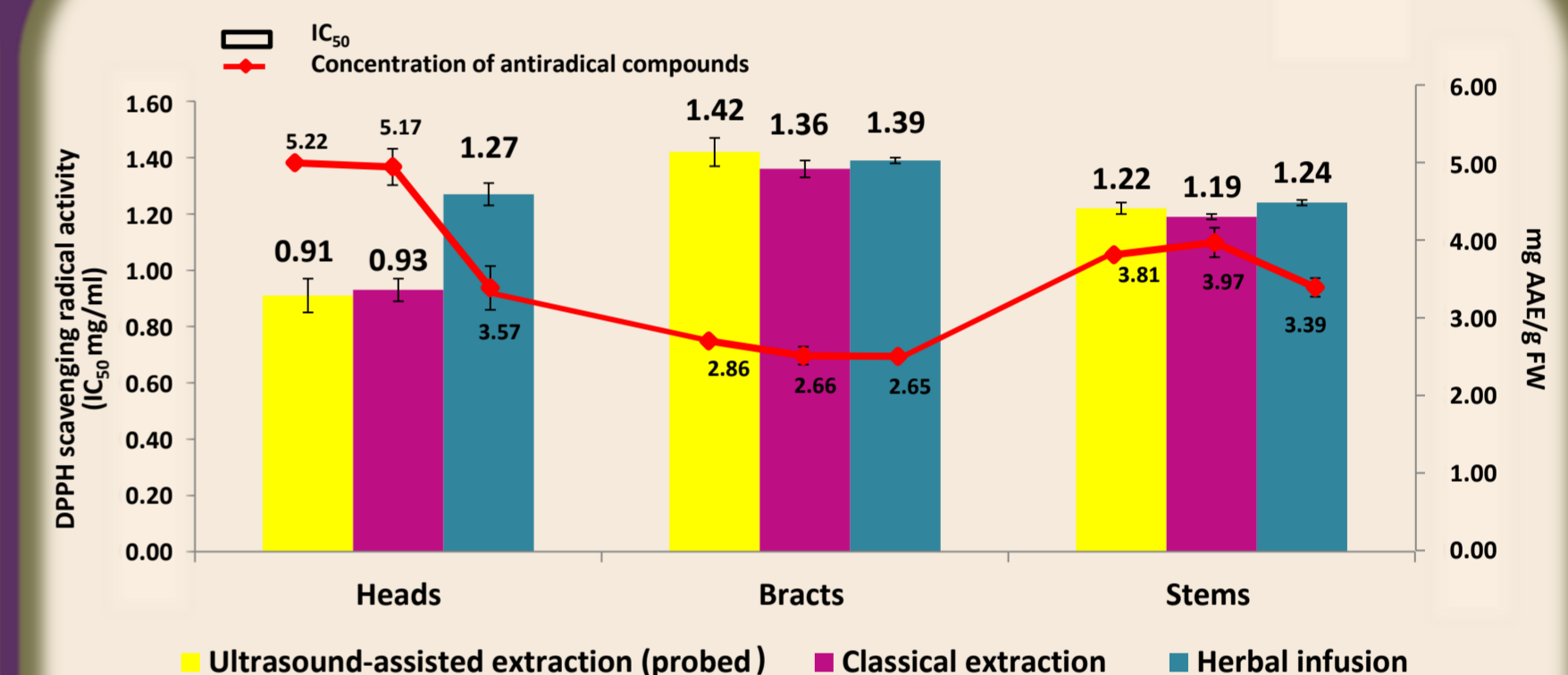


Figure 2: Antioxidant activity of *C.cardunculus* L. extracts (heads, bracts, stems) from different extraction methods.

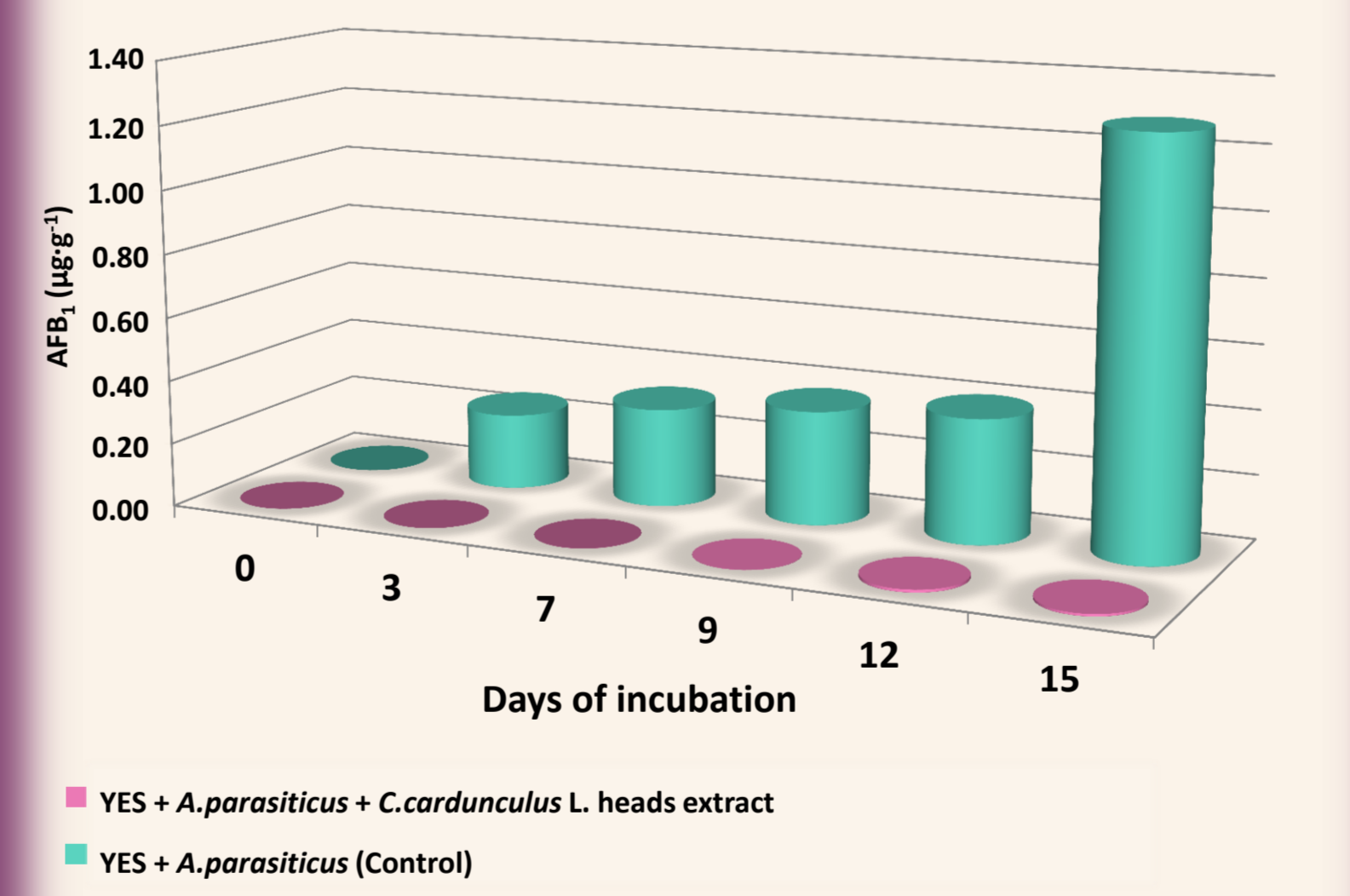
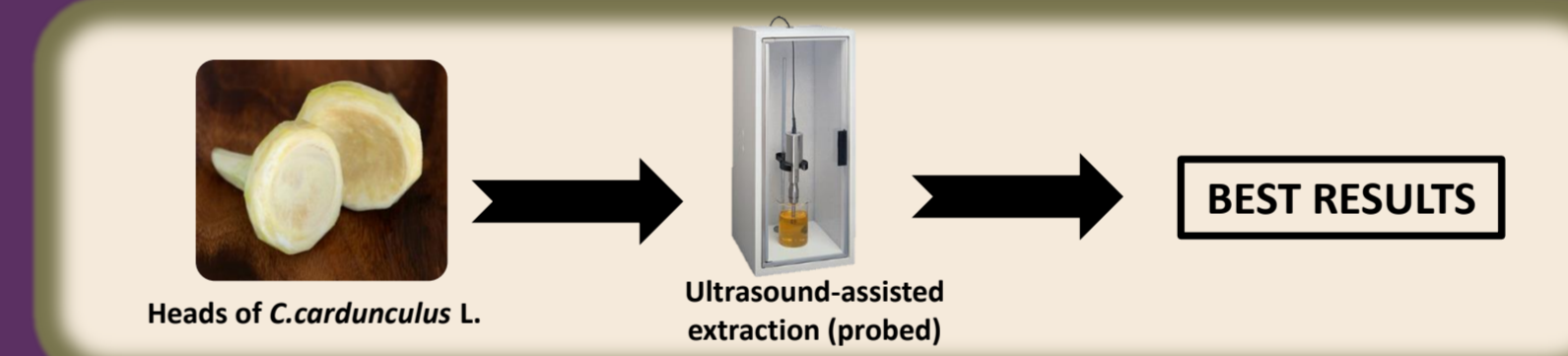


Figure 3: Comparison of AFB₁ production in: a) YES inoculated with *A.parasiticus* + addition of 50µl of *C.cardunculus* L. heads extract (c=1g/ml), b) YES inoculated with *A.parasiticus* (control)

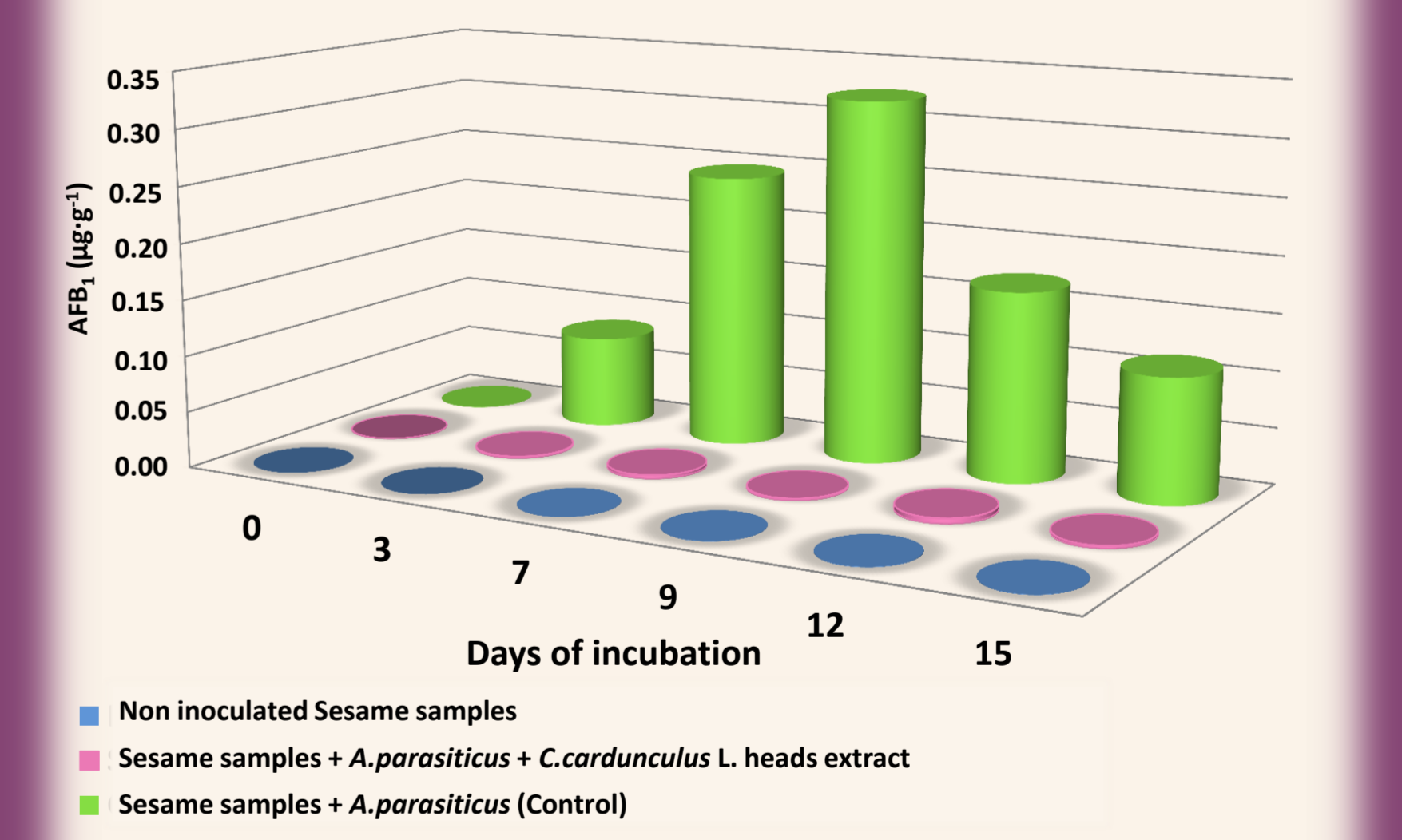


Figure 4: Comparison of AFB₁ production in: a) sesame samples non inoculated, b) sesame samples inoculated with *A.parasiticus* (control), c) sesame samples inoculated with *A.parasiticus* + addition of 50µl of *C.cardunculus* L. heads extract (c=1g/ml)

CONCLUSIONS

- From the applied extraction techniques, the ultrasound-assisted extraction showed better results as far as concern the antioxidant activity and total phenolic content (TPC). (Fig.1-2)
- C. cardunculus* L. head extract (ultrasound-assisted extraction) showed maximum TPC and the highest DPPH[•] scavenging activity in comparison with bracts and stems. (Fig.1-2)
- All the concentrations (0.005-1g/ml) of *C. cardunculus* L. head extract, inhibited the *A. parasiticus* mycelial growth. However, concentration of 1g/ml, displayed the most significant antifungal activity (percentage of inhibition → 42.1%).
- The recovery of the method for AFB₁ determination in sesame seed was found to be 111.5% (RSD%= 5.09), while the detection limit (DL) and quantification limit (QL) were 0.02 ng·g⁻¹ and 0.2 ng·g⁻¹ respectively.
- AFB₁ production in YES inoculated with *A. parasiticus* and addition of *C. cardunculus* L. head extract, was significantly lower (99.4%) compared to AFB₁ production by *A. parasiticus* in YES medium (control). (Fig.3)
- AFB₁ production in sesame seeds inoculated with *A. parasiticus* and addition of *C. cardunculus* L. head extract, was significantly lower (99.6%) compared to AFB₁ production by *A. parasiticus* in sesame seeds (control). (Fig.4)
- AFB₁ production in sesame seeds non inoculated, without addition of *C. cardunculus* L. head extract, was found at low levels (0.13-0.47 ng·g⁻¹).

DUE TO ITS ANTIFUNGAL AND ANTI-AFLATOXIGENIC EFFECTIVENESS, *C. CARDUNCULUS* L. CAN BE USED FOR PRE- AND POSTHARVEST AF CONTROL STRATEGIES OR DURING STORAGE

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