UNIVERSITY SIDI MOHAMED BEN ABDELLAH

FACULTY OF SCIENCE

FEZ

CHEMISTRY DEPARTMENT

In vitro antioxidant activity, phytochemical screening and total phenolic and flavonoid contents from the leaves extracts of *Stachys germanica subs cordigera briq*

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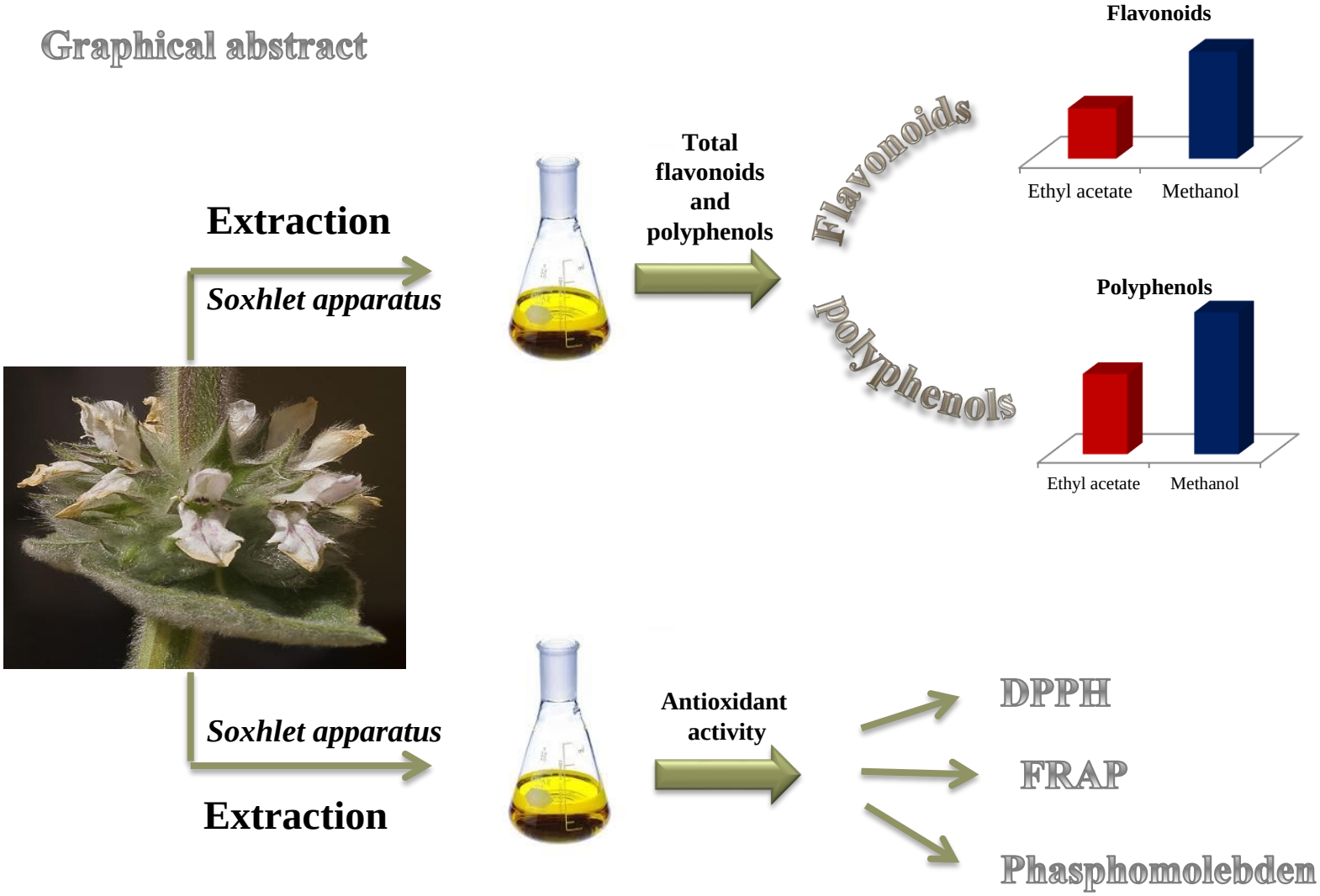
In vitro antioxidant activity, phytochemical screening and total phenolic and flavonoid contents from the leaves extracts of *Stachys germanica subs cordigera briq*

Graphical abstract



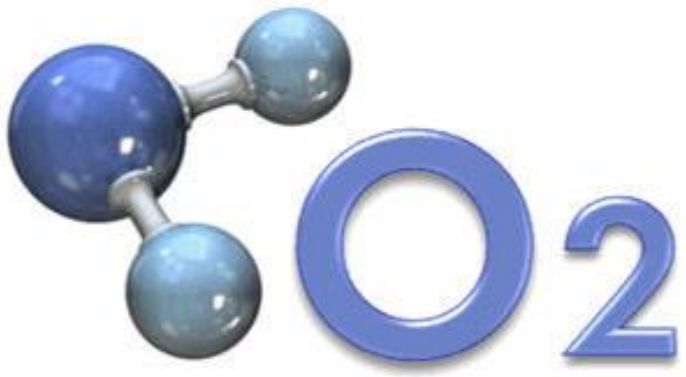
Abstract

This study is planned to perform phytochemical screening, evaluate antioxidant activity and assess total phenolics and flavonoids content of methanolic and ethyl acetate extracts from the leaves of *Stachys germanica subs cordigera briq*. The dried powdered leaves of *Stachys germanica subs cordigera briq* (80 g) were extracted exhaustively by Soxhlet apparatus *with increasing polarity of solvents (hexane, ethyl acetate and methanol)*. The total phenolic and flavonoid contents in the methanolic and ethyl acetate extracts were determined by using the Folin-Ciocalteu reagent and aluminum chloride method, respectively. The antioxidant activities were examined by three different methods, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, reducing power scavenging activity (FRAP) and total antioxidant capacity. Phytochemical analysis of all extracts showed the presence of major classes of phytochemicals such as, flavonoids, tannins and polyphenols. Total phenolic and flavonoid contents results are showed in a large dominance in ethyl acetate extract. In vitro antioxidant activities of both extracts (ethyl acetate and methanol) were significant and ethyl acetate extract showed a higher potency than reference antioxidant Butylated Hydroxy Toluene (BHT) in total antioxidant capacity essay. It can be concluded that the crude extracts from the leaves of *Stachys germanica subs cordigera briq*. are a potential source of natural antioxidants which can be used in preventing the progression of many diseases.

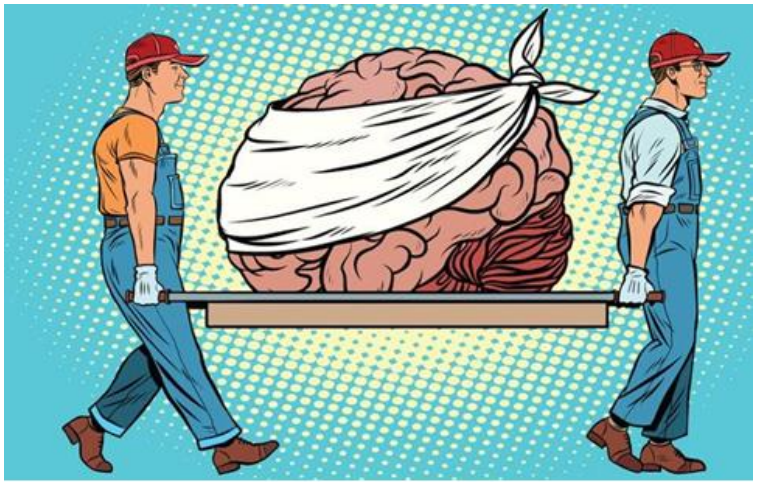
Keywords: ***Stachys germanica subsp cordigera briq*, phytochemical screening, total phenolics and flavonoids content, antioxidant activity**

OUTLINE

- Introduction
- Objectives
- Materials and Methods
- Results
- Conclusion

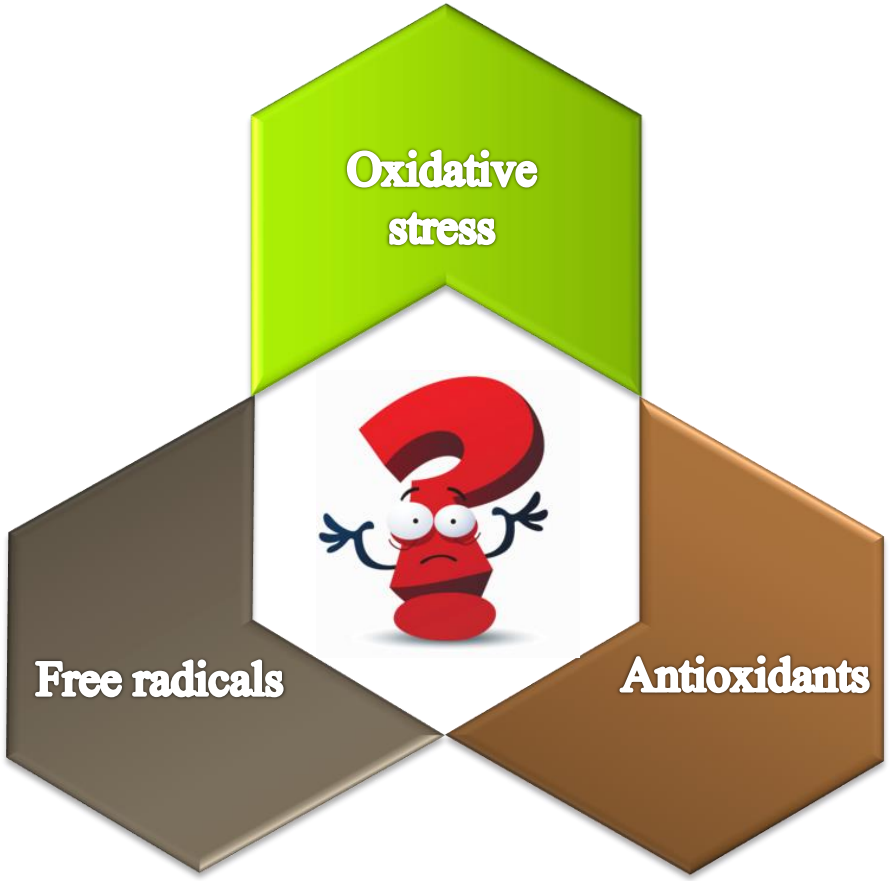


humans, along with many other creatures, need oxygen in the air we breathe to stay alive

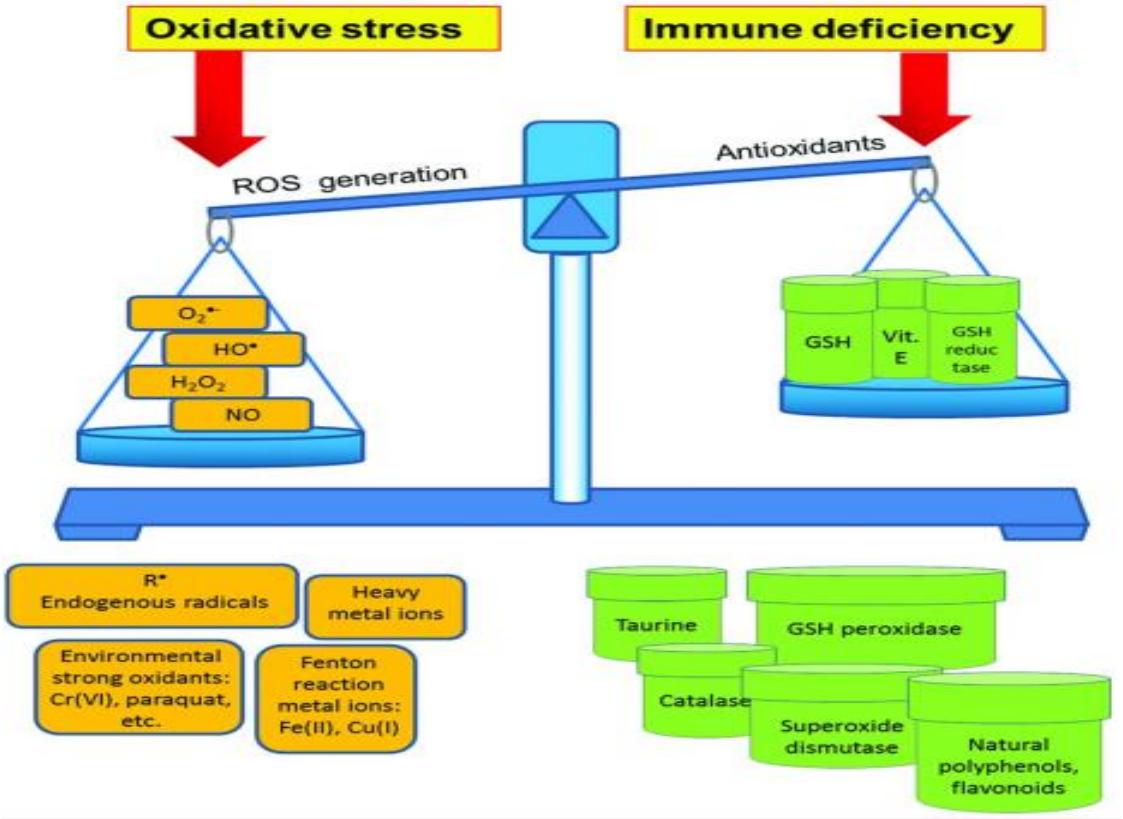


highly reactive atom that is capable of becoming part of potentially damaging molecule commonly called ''free radical''

The excessive production of these free radicals creates what is known as oxidative stress.

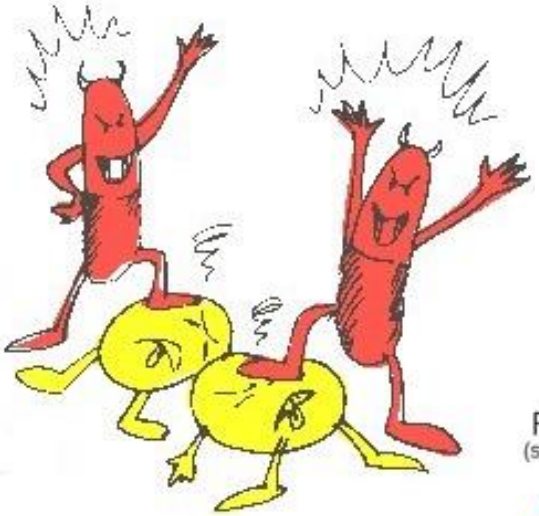


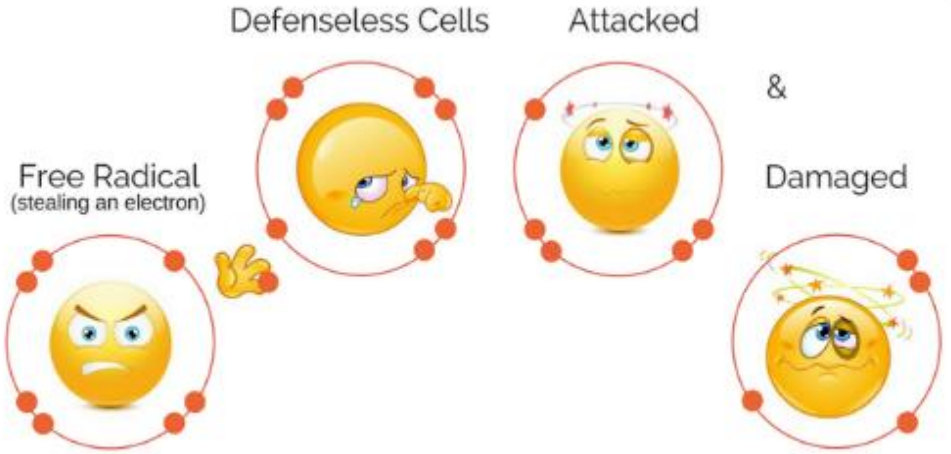
Oxidative stress



Free radicals

What are Free Radicals?

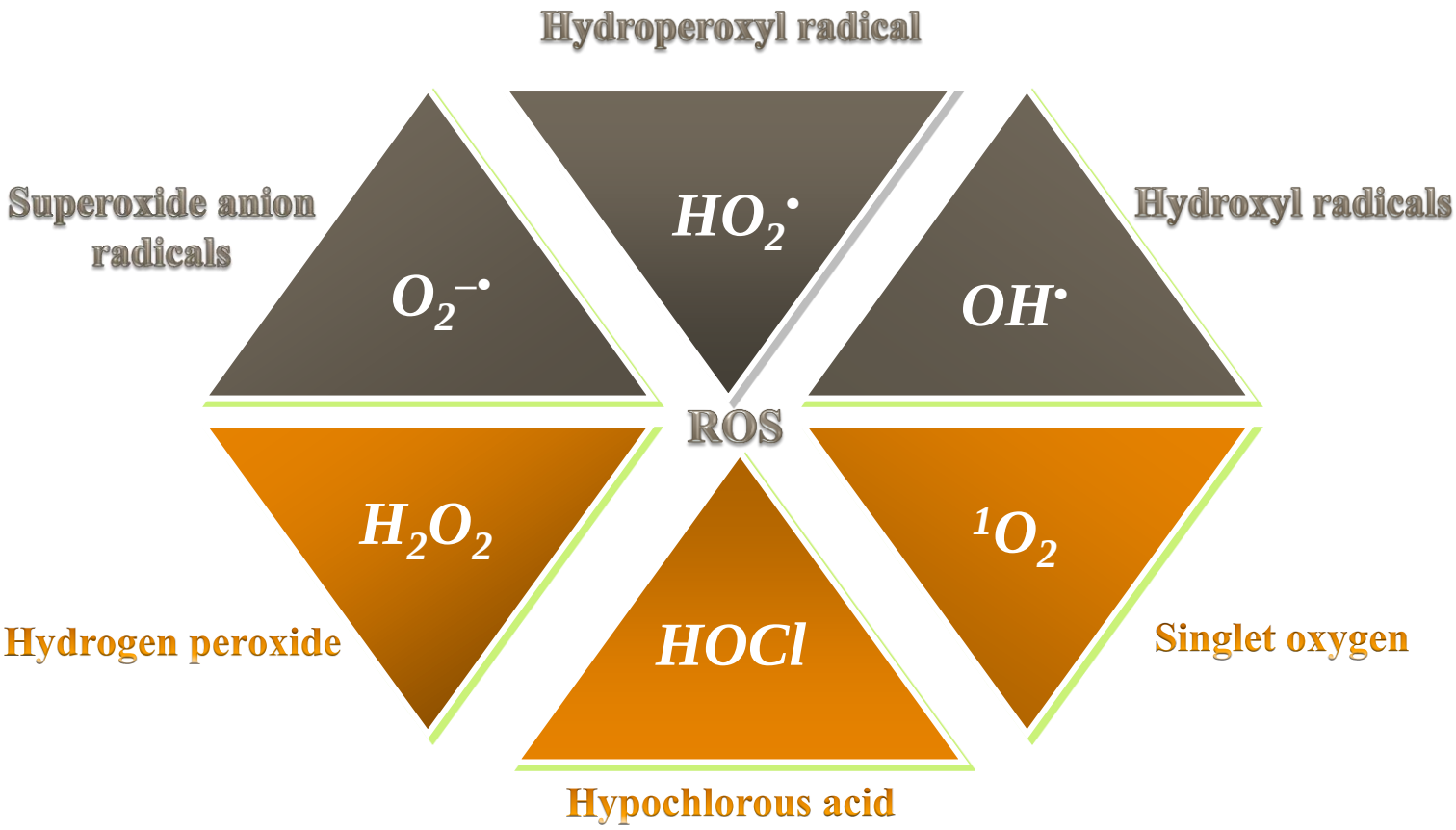




Free radicals

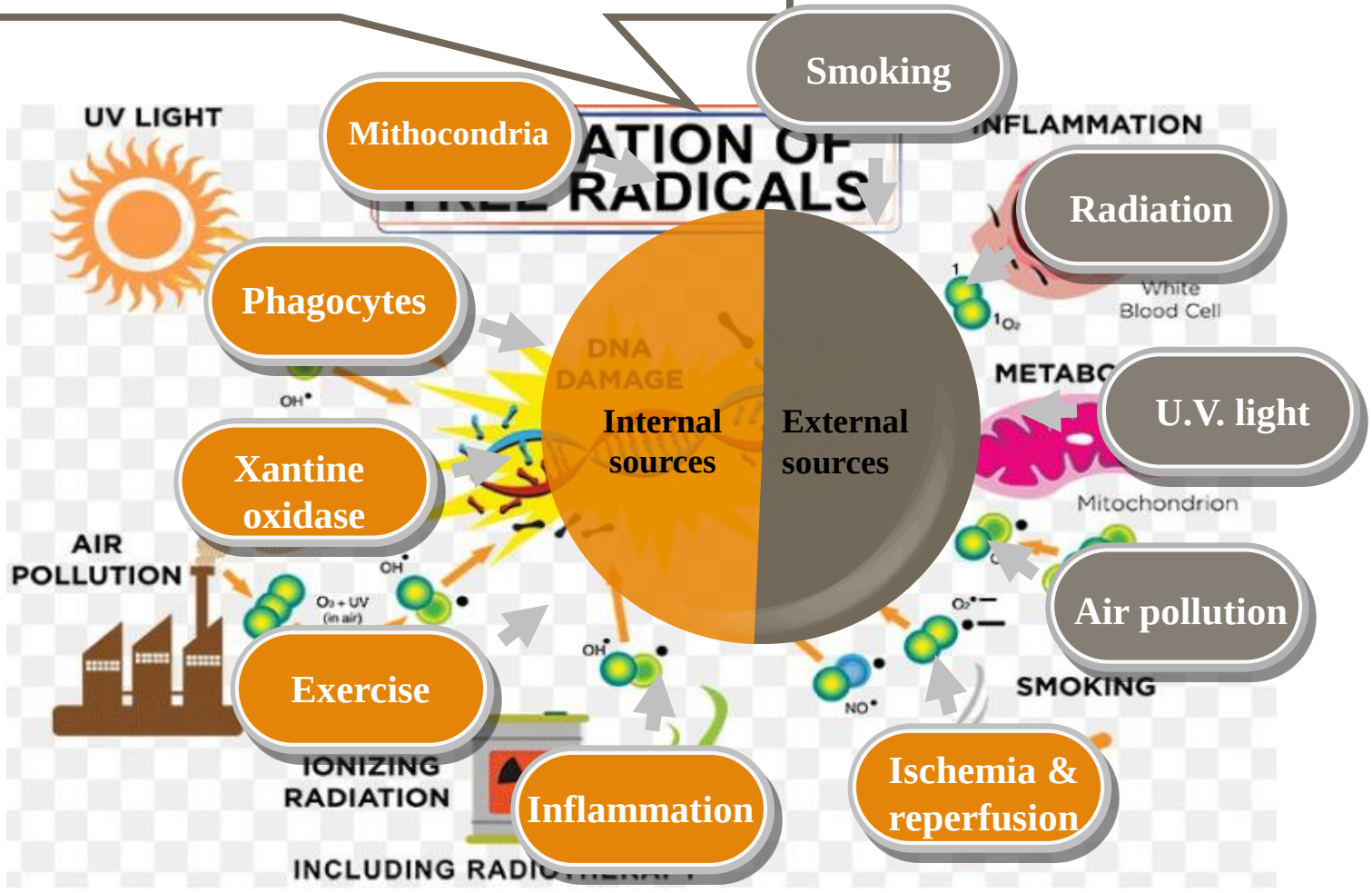
- Free radicals are capable of attaching cells of body, causing them to lose their structure and function.
- Although the initial attack cause the free radical to become neutralized, another free radical is formed in this process, causing a chain reaction is occur.
- And until subsequent free radicals are deactivated, thousands of free radical reaction can occur within second of initial reaction.
- Free radicals have been implicated in the pathogenesis of at least 50 diseases cardiovascular diseases, cancers, neurodegenerative diseases, Alzheimer's disease inflammatory diseases and other related diseases

Free radicals



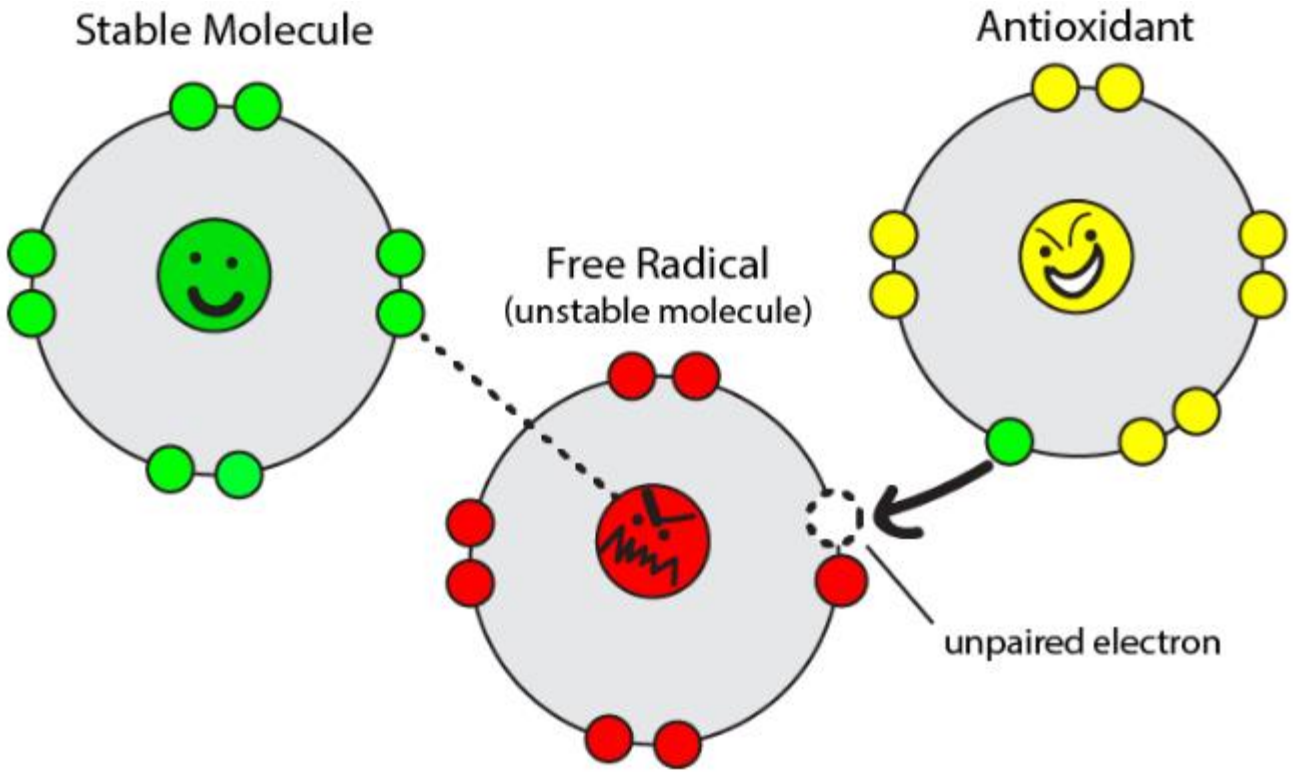
Source of free radicals

Free radicals and other ROS are derived either from endogenous metabolic process in the human body (Internal source) or from external sources



Antioxidants

Antioxidants are chemical substances that donate an electron to the free radical and convert it to harmless molecule.



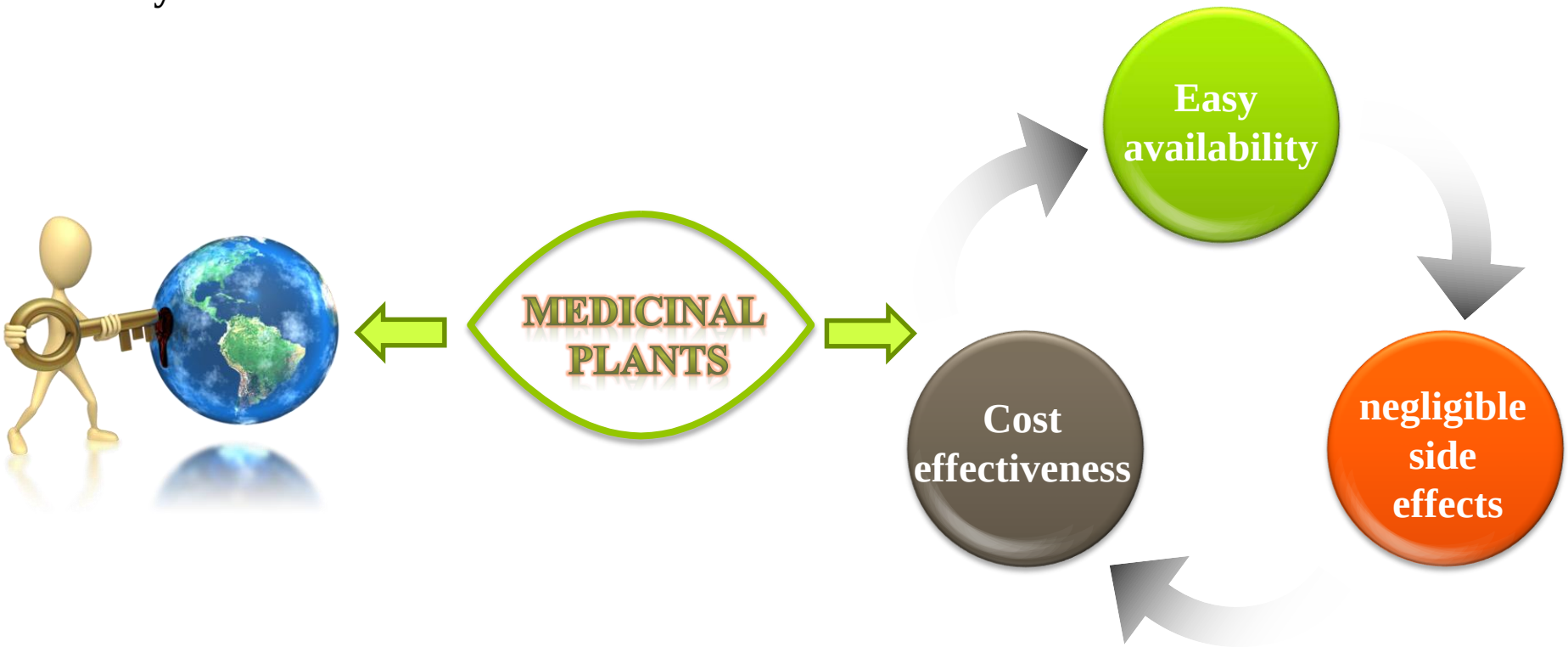
Selection cretiria of antioxidants

Following criteria should be considered while selecting an antioxidant

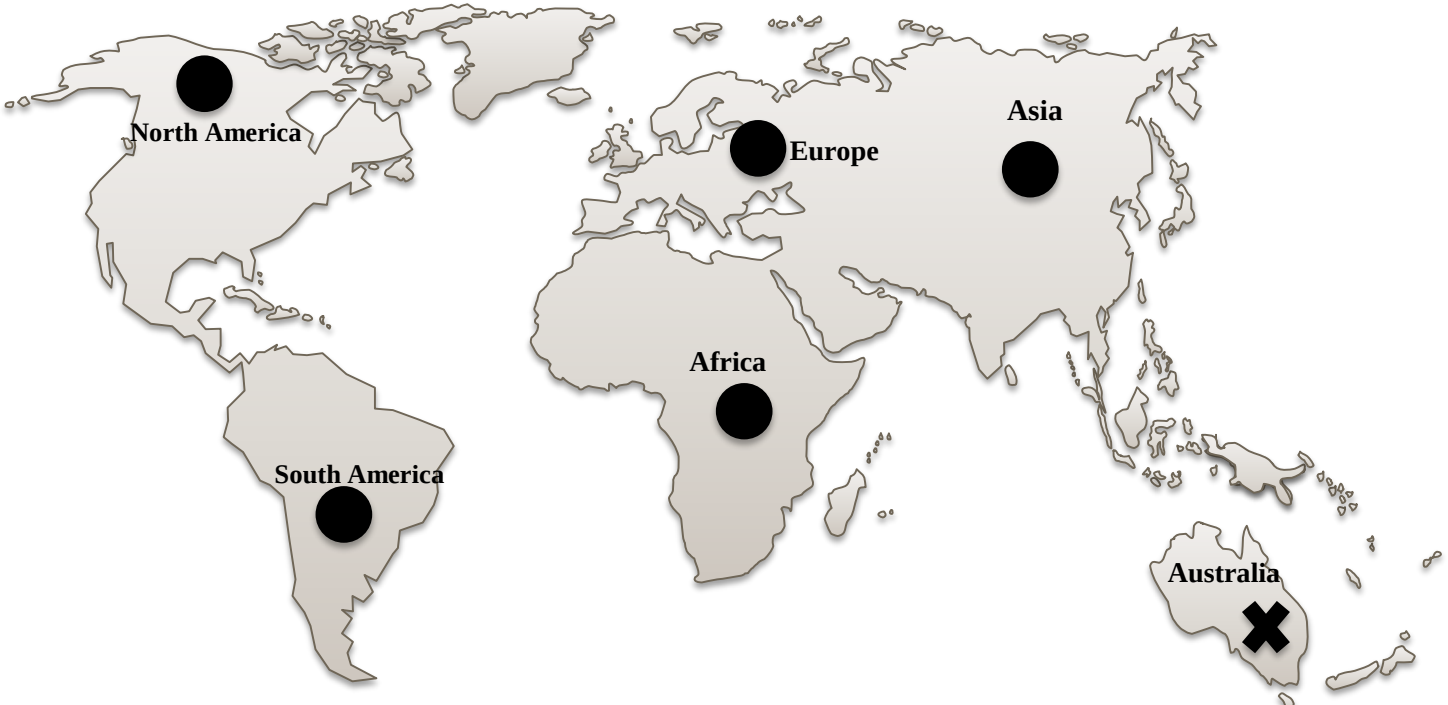
- **It should be able to produce desire redox reaction**
- **It should be physiologically and chemically compatible**
- **It should be physiologically inert**
- **It should be non-toxic both in the reduced and oxidized forms**
- **It should be effective in low concentration**
- **It should be provide prolonged stability to the formulation**

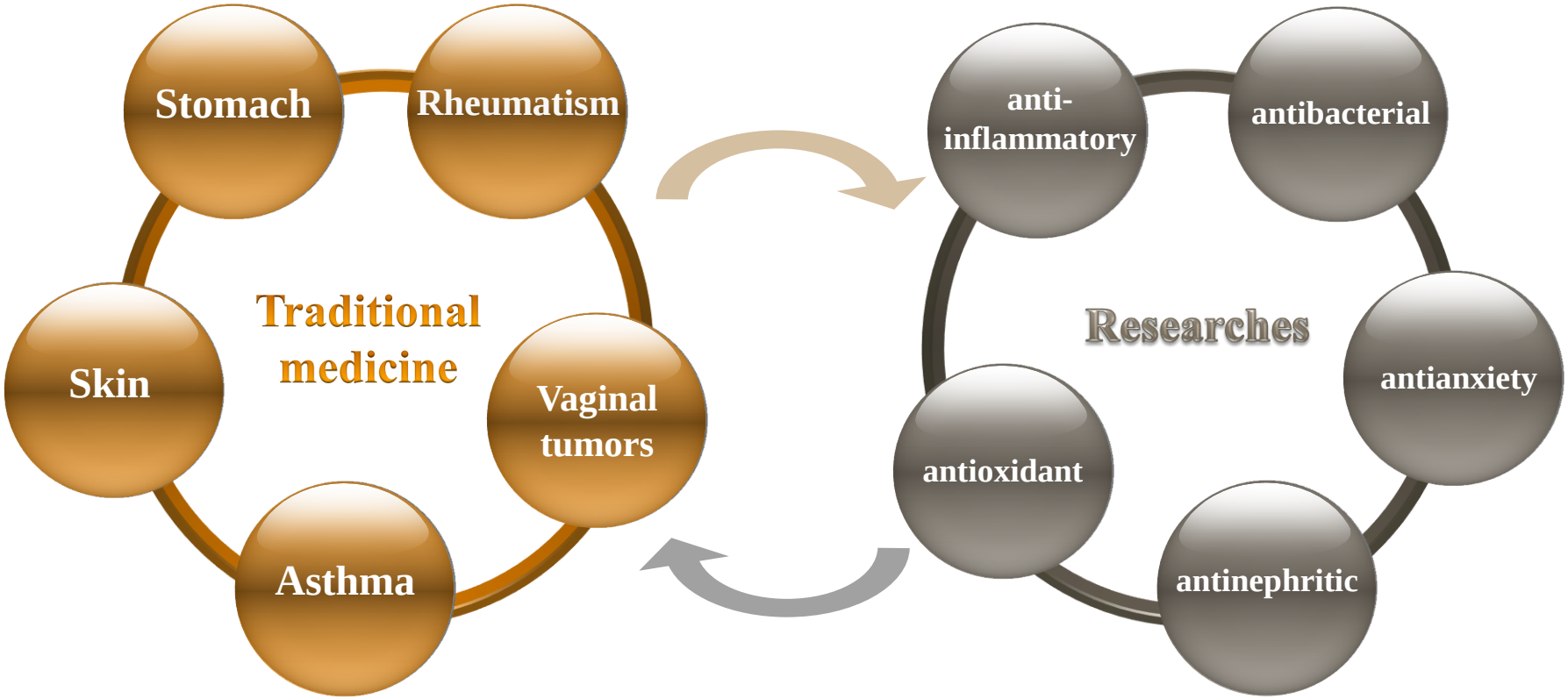
MEDICINAL PLANT

Natural compounds derived from medicinal plants play a central part in the health care and drug development in classical as well as advanced systems of medicine.



Stachys germanica subs cordegira briq





Many *Stachys* species are used in the preparation of food such as yoghurt or jelly to improve the taste and as flavors and seasoning

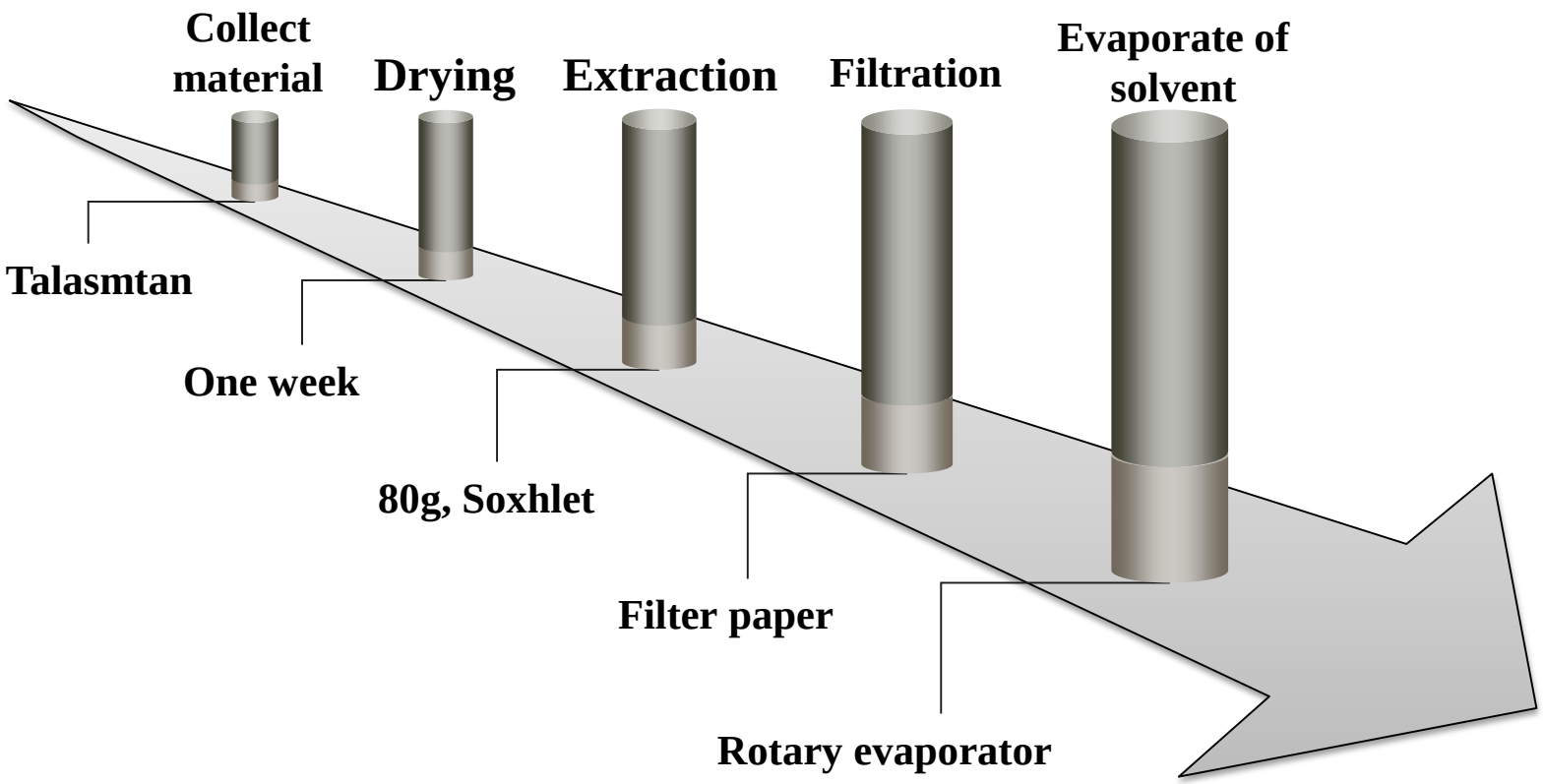
Aims and Objectives

The present investigation is undertaken by utilizing the plant *Stachys germanica subs cordegira briq* with following objectives:

- **Extraction of the leaves with different solvents**
- **Preliminary phytochemical analysis**
- **Determination of total phenolic and flavonoid contents at the different extracts**
- **Evaluation the antioxidant activities**

Material and methods

1-COLLECTION OF MATERIAL AND EXTRACTION PROCEDURE



2- PHYTOCHEMICAL ANALYSIS

- Sterols and terpenes: Burchard's test
- Polyphenols: $Fecl_3$ solution
- Flavonoids: cyanidine reaction
- Tannins: Stiasny's test
- Alkaloids: Dragendorff's test

3-TOTAL FLAVONOIDS CONTENT

Total flavonoid content was determined spectrophotometrically by using the $AlCl_3$ reagent as described by Dewanto et al 2002

250µl of crude extract + 75µl of 5% $NaNO_2$

↓ **6min Incubation at room temperature**

Add 150µl of 10% $AlCl_3$

↓ **5min Incubation at room temperature**

Add 0.5 mL of 1M NaOH

↓

Adjust volume with distilled water to 2.5mL

Absorbance at 510 nm with U.V visible spectrophotometre

TOTAL PHENOLIC CONTENT

Total phenolic content was determined spectrophotometrically by using Folin-Ciocalteu method as described by El Hajaji et al 2012

100µl Folin-ciocalteu reagent + 1.58mL distilled water + 20µl crude extract

↓ **5min**
Incubation at room temperature

Add 300µl of Na_2CO_3(25%)

↓ **60min**

Measuring of TPC at 765nm

ANTIOXIDANT ACTIVITIES

Antioxidant activities were examined by three different methods namely,

- 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity
- Reducing power scavenging activity (FRAP)
- Total antioxidant capacity.

❖ 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The leaves extracts of *S.germanica subs cordigera briq* were tested in their free radical scavenging activity using (DPPH), according to the protocol described by El Hajaji et al 2012.

100µl of extract + 10mL of methanolic solution of DPPH

↓

Incubation at room temperature for 30 min

↓

Record the absorbance at 517 nm

Inhibition ratio : $\% \text{ inhibition} = [(A_0 - A_1)/ A_0] \times 100$

A_0: **absorbance of control reaction ;** A_1: **absorbance of test compounds**

BHT : positive control. The test was carried out in triplicate

❖ Reducing power scavenging activity (FRAP)

The reducing power was determined according to the protocol described by El Hajaji et al 2012.

Extract at various conc + 0.2M phosphate buffer (pH 6.6) + ($K_3Fe(CN)_6$) (1%)

↓ Mix and incubate at 50 ^{0}C in water bath for 20min

Add $C_2HCl_3O_2$ (10%)

↓

The upper layer mixed with DW and of FeCl3 (1%)

↓

Absorbance at 700nm

Ascorbic acid : standard

❖ Total antioxidant capacity.

The total antioxidant capacity of the extracts was evaluated by using the phosphomolybdenum method as montioned by El Hajaji et al 2012

- **The test is based on the reduction of Mo (VI) to Mo(V) in the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH.**
- **The absorbance of the solution was measured at 695 nm using a U.V.visible spectrophotometer**
- **The antioxidant capacity of each sample was served as Ascorbic Acid equivalent using the following linear equation in using ascorbic acid as standard: [A= 0.0037C + 0.0343 ; R^2=0.991]**

A: absorbance at 695 nm ; C: concentration as ascorbic acid equivalent (µg/ml).

The values represent the triplicate analysis.

Results

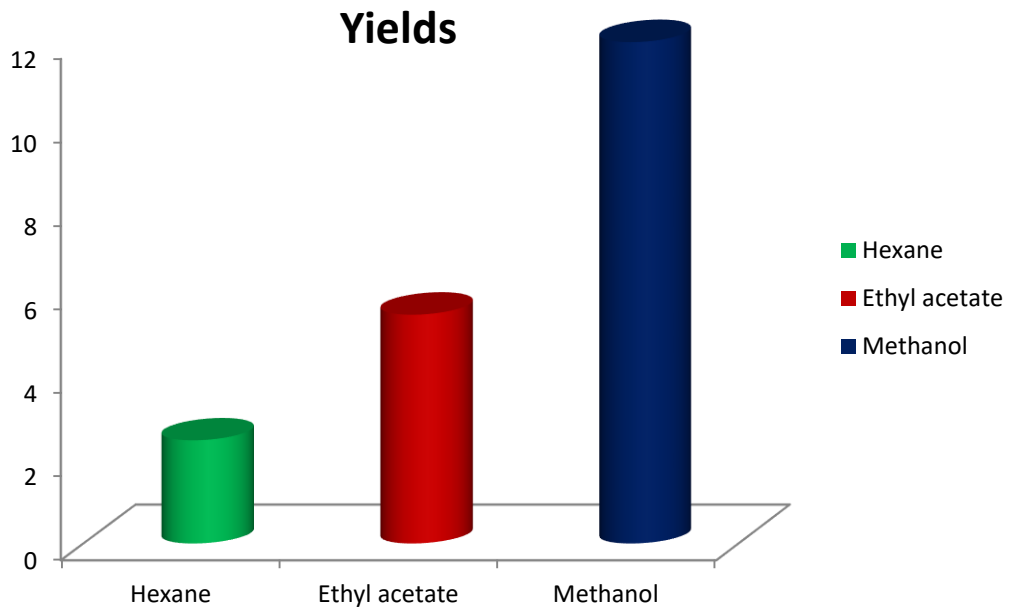


***Figure 1*. Yields of hexane, ethyl acetate and methanol extracts of *Stachys germanica subs cordigera briq*.**

Results

Preliminary phytochemical analysis from the leaves extracts of *Stachys germanica subsp cordigera briq* showed the presence of major classes of secondary metabolites.

Extracts / Metabolites	Hexane	Ethyl acetate	Methanol
Polyphynols	-	+	+
Sterols/steroids	+	-	-
Terpenes/Terpenoids	+	-	-
Tannins	-	+	-
Alkaloids	-	-	-
Flavonoids	-	+	+

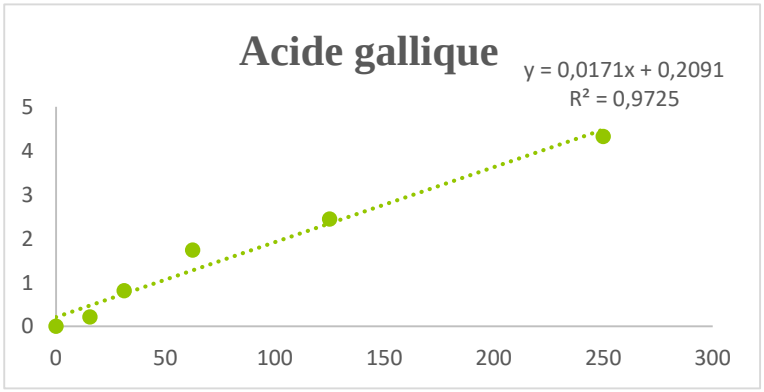
Table. 1 : Results of preliminary phytochemical screening of *S.germanica subsp cordigera briq*. leaves extracts.

Results

TOTAL PHENOLIC CONTENTS

Total phenolic content was expressed as GAE using the following linear equation as standard: y= 0.0171x+0.2091, R^2=0.975.

Ethyl acetate extract = 23.2 µg/mL GAE ; Methanol extract = 15.7 µg/mL GAE



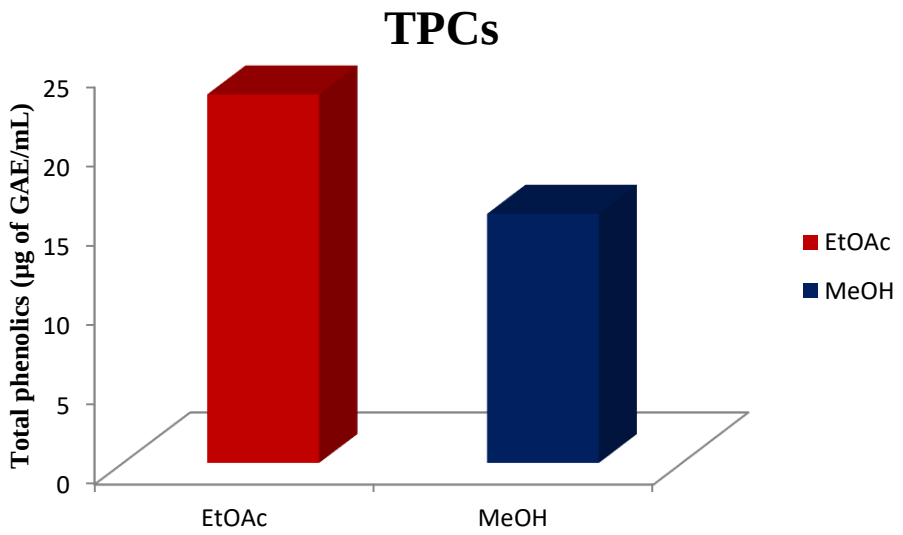


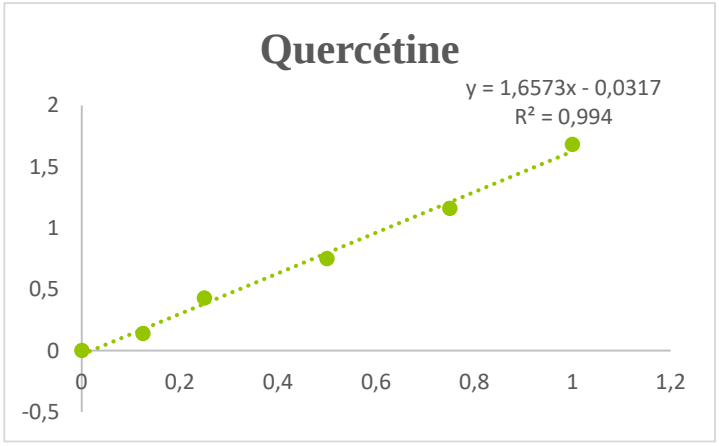
Fig 2. Total phenolics contents of ethyl acetate and methanol extracts of *S. germanica subs cordigera briq*.

Results

TOTAL FLAVONOIDS CONTENTS

Total flavonoids contents, they are made as quercetin equivalent using also the following linear equation with quercetin as standard: y= 1.657x+0.0317; R^2=0.994.

Ethyl acetate extract = 82 µg/ml QE ; Methanol extract =42µg/ml QE.



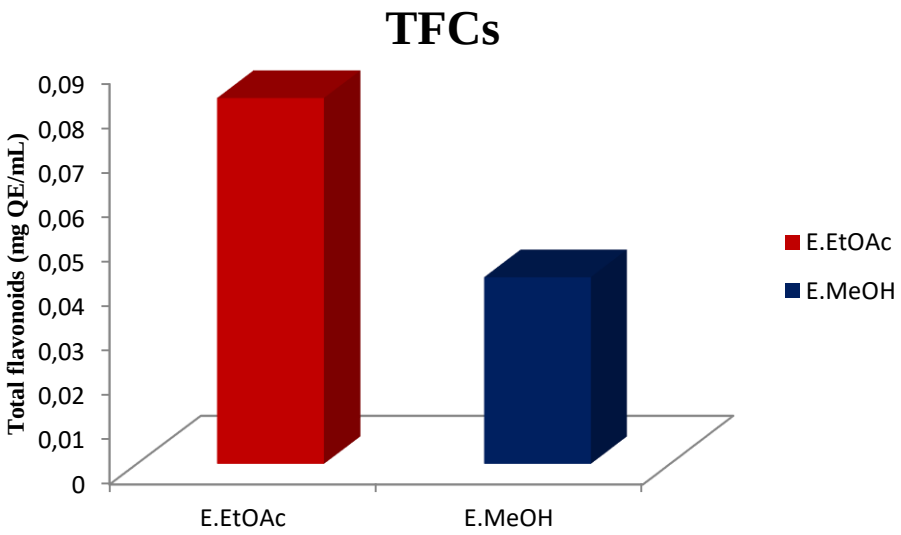


Fig 3. Total Flavonoid contents of ethyl acetate and methanol extracts of *S. germanica subs cordigera briq*.

Results

DPPH ANTIOXIDANT ACTIVITY

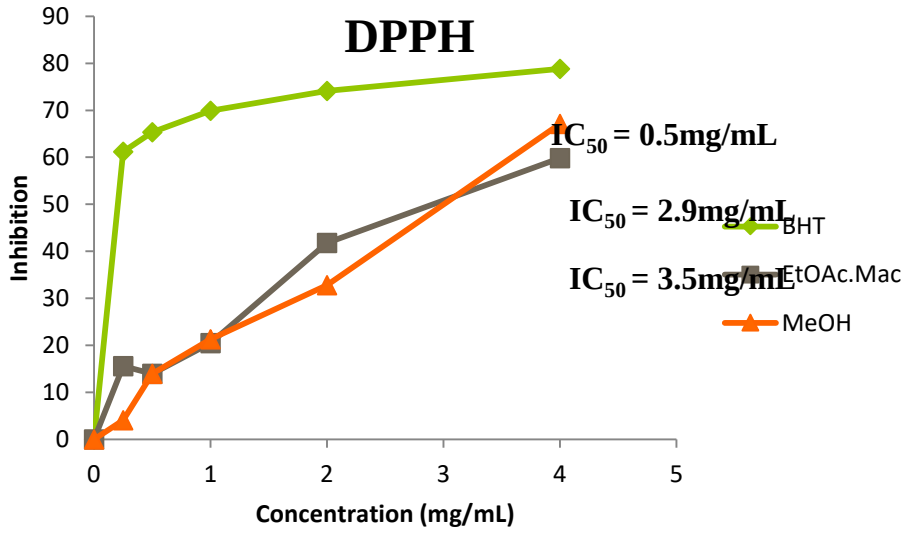


Fig 4. Antioxidant activity of *Stachys germanica subs cordigera briq*. leaves extracts against DPPH

Results

FERRIC REDUCING ANTIOXIDANT POWER

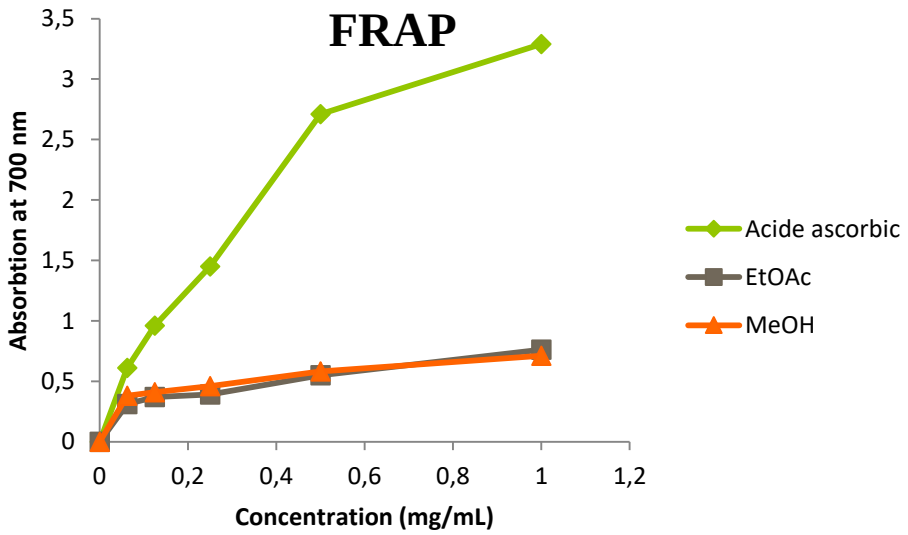


Fig 5. Reducing power of of *Stachys germanica subs cordigera briq*. leaves extracts

Results

TOTAL ANTIOXIDANT CAPACITY

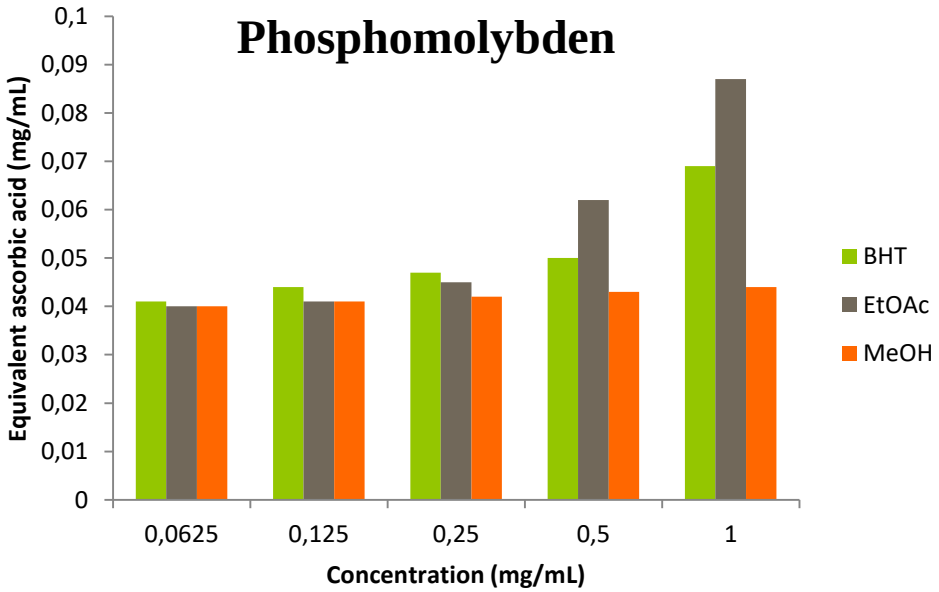


Fig 6. Antioxidant capacity of *Stachys germanica subs cordigera briq.* leaves extracts

Conclusion

- **Phytochemical screening showed the presence of various classes of bioactive chemical constituents in all extracts of *Stachys germanica subs cordigera briq* including sterols/steroids, terpenes /terpenoids, polyphenols, tannins and flavonoids.**
- **Total phenolic and flavonoid contents results showed a large dominance in ethyl acetate extract.**
- **The leaves extracts from *Stachys germanica subs cordigera briq* showed a significant antioxidant activity as well as, ethyl acetate extract exhibited greater antioxidant than BHT as measured by total antioxidant capacity.**
- **These results suggested that the leaves extracts from *Stachys germanica subs cordigera briq.* can be used as possible in natural antioxidant source. It is then necessary to identify and isolate the compounds that are responsible to these antioxidant activities.**

Thank you for you attention