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Determination of total phenolics and flavonoids content and evaluation of antioxidant activity of *Tomborissa comorensis* fruit

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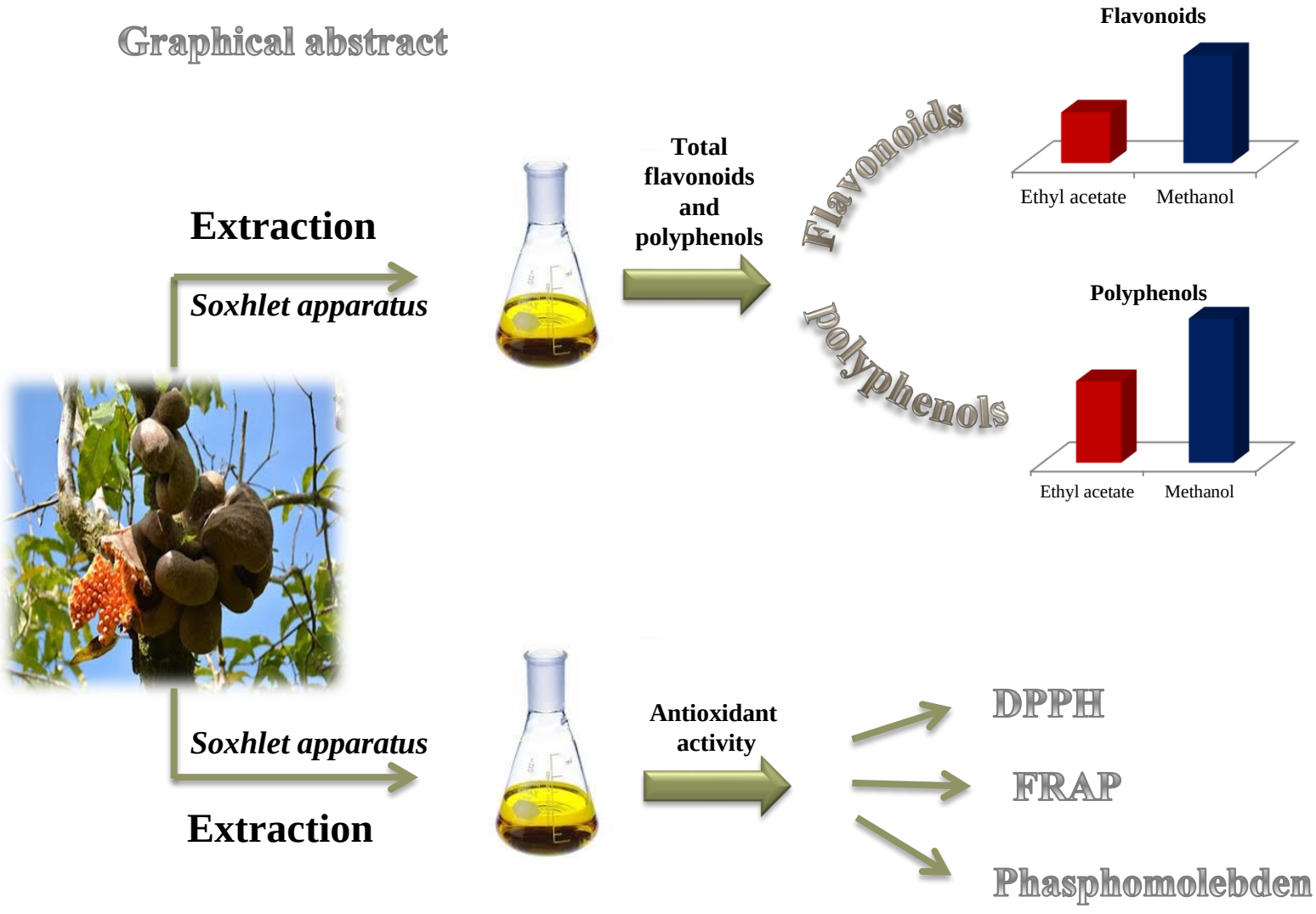
Determination of total phenolics and flavonoids content and evaluation of antioxidant activity of *Tomborissa comorensis* fruit

Graphical abstract



Abstract

The objective of this study was to perform phytochemical screening, estimate total phenolics, flavonoids and to evaluate antioxidant potential of *Tomborissa comorensis* fruit. The dried and pulverized fruit of *Tomborissa comorensis* (150g) were extracted exhaustively by Soxhlet with increasing polarity of solvents (hexane, ethyl acetate and methanol). Folin-Ciocalteu reagent and aluminium chloride colorimetric methods were used to estimate total phenolic and flavonoid content of extracts. Three different methods namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, reducing power scavenging activity (FRAP) and total antioxidant capacity were used to determine *in vitro* antioxidant activity. Phytochemical screening concerns the presence of flavonoids in the ethyl acetate and methanol extracts and tannins only on the methanol extract. Total phenolic and flavonoids contents results are showed in a large dominance in methanol extract. All tests showed significant dose dependent antioxidant activities. The ethyl acetate extract shows the high activity in DPPH radical scavenging activity but in reducing power assay, it's the methanol extract which manifested the high activity. The results of this study show that the fruit of *T. comorensis* is a rich source of phenolic compounds that can play an important role in preventing the progression of many diseases.

Keywords: *Tomborissa comorensis*, antioxidant activity, phenolic content, Flavonoid content

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OUTLINE

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

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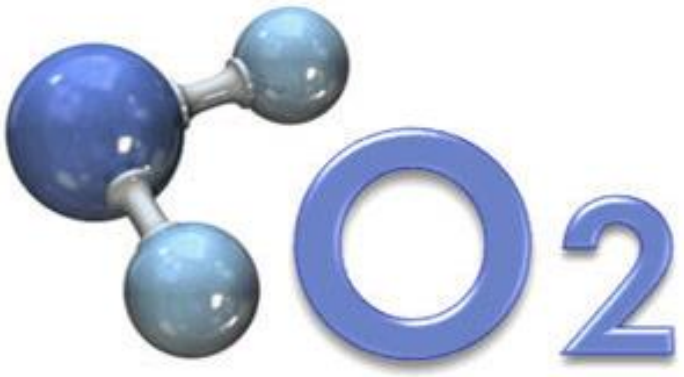
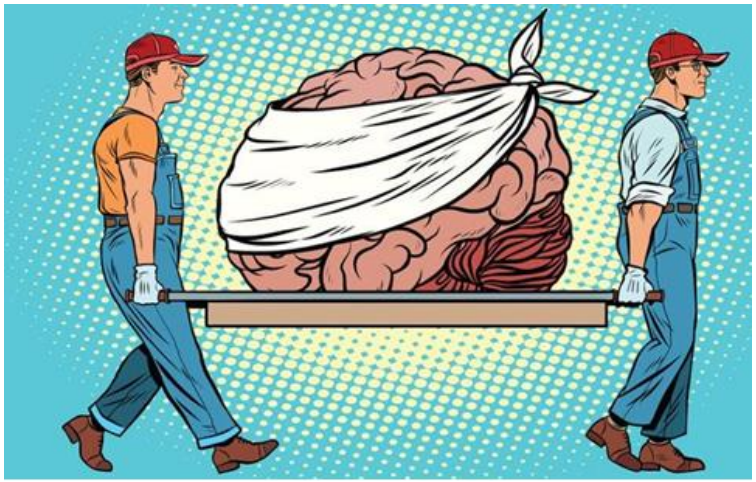
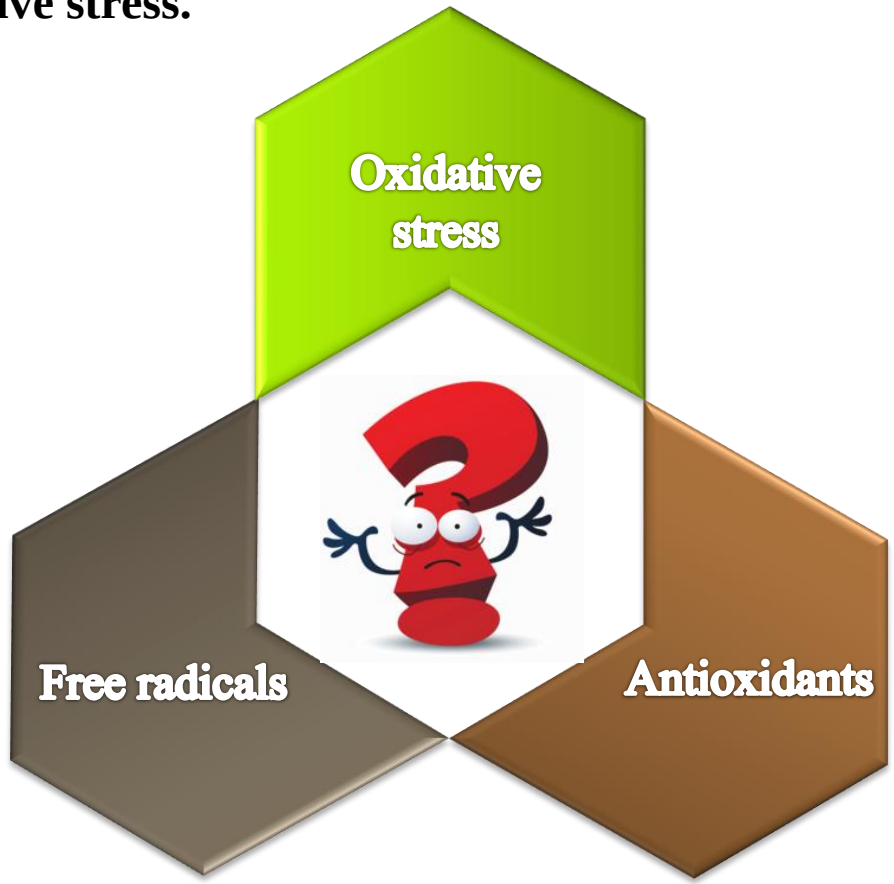


humans, along with many other creatures, need oxygen in the air we breathe to stay alive

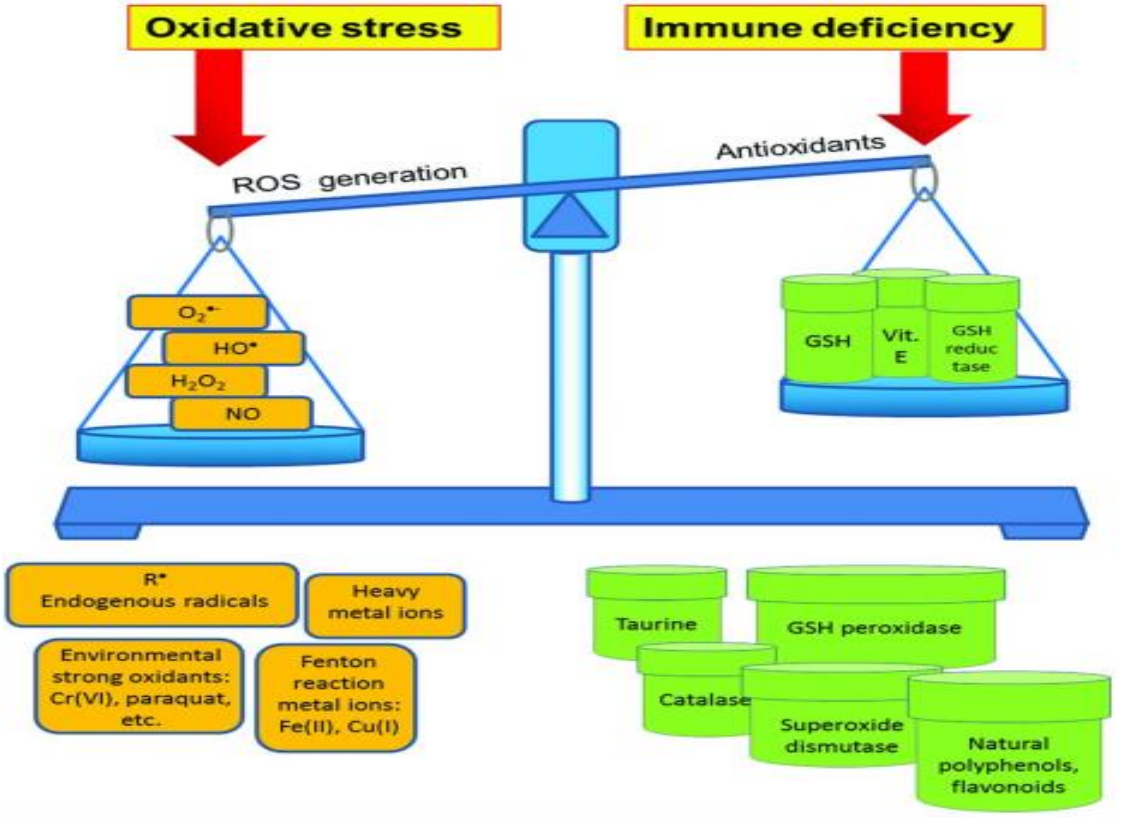


highly reactive atom that is capable of becoming part of potentially damaging molecule commonly called ''free radical''

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



Oxidative stress



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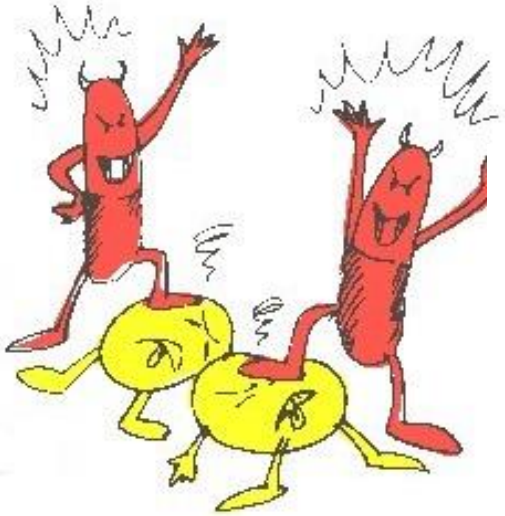
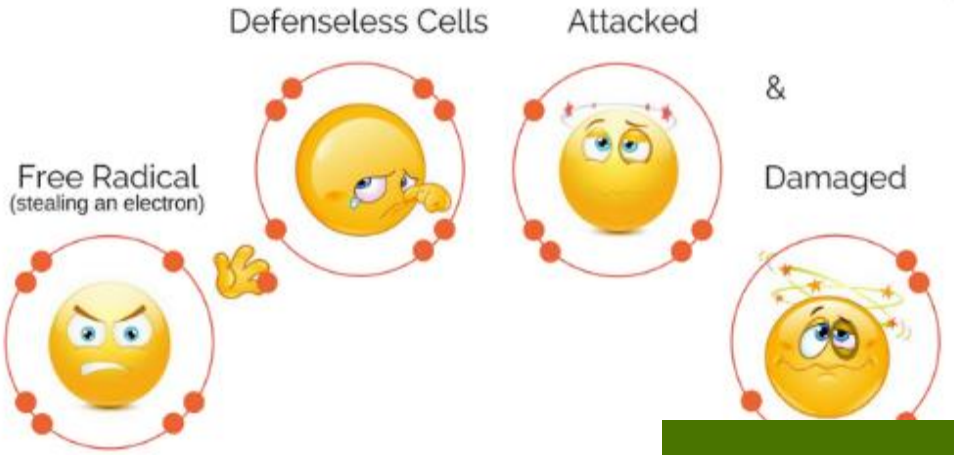
Free radicals

What are Free Radicals?

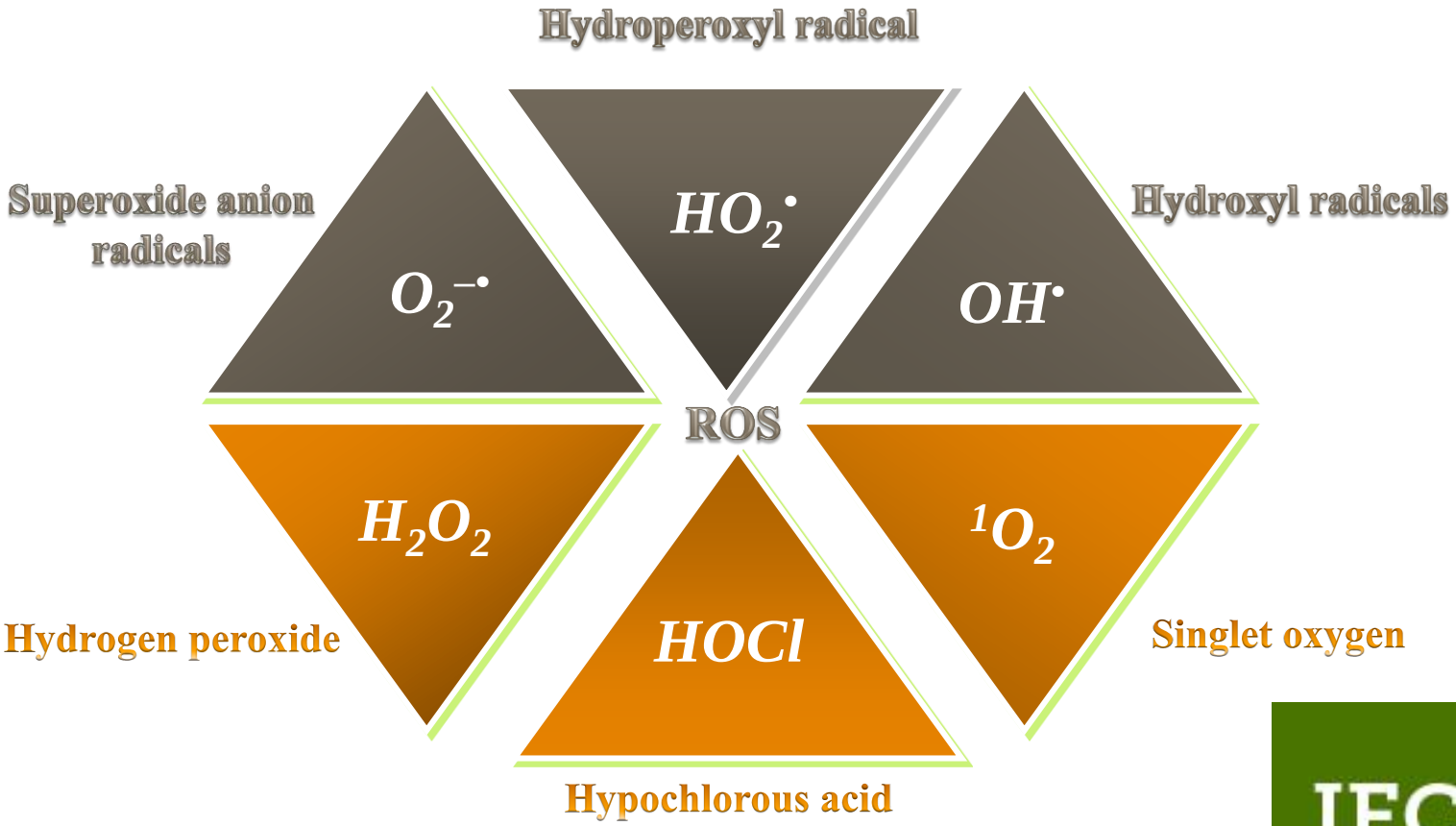




Free radicals

- 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



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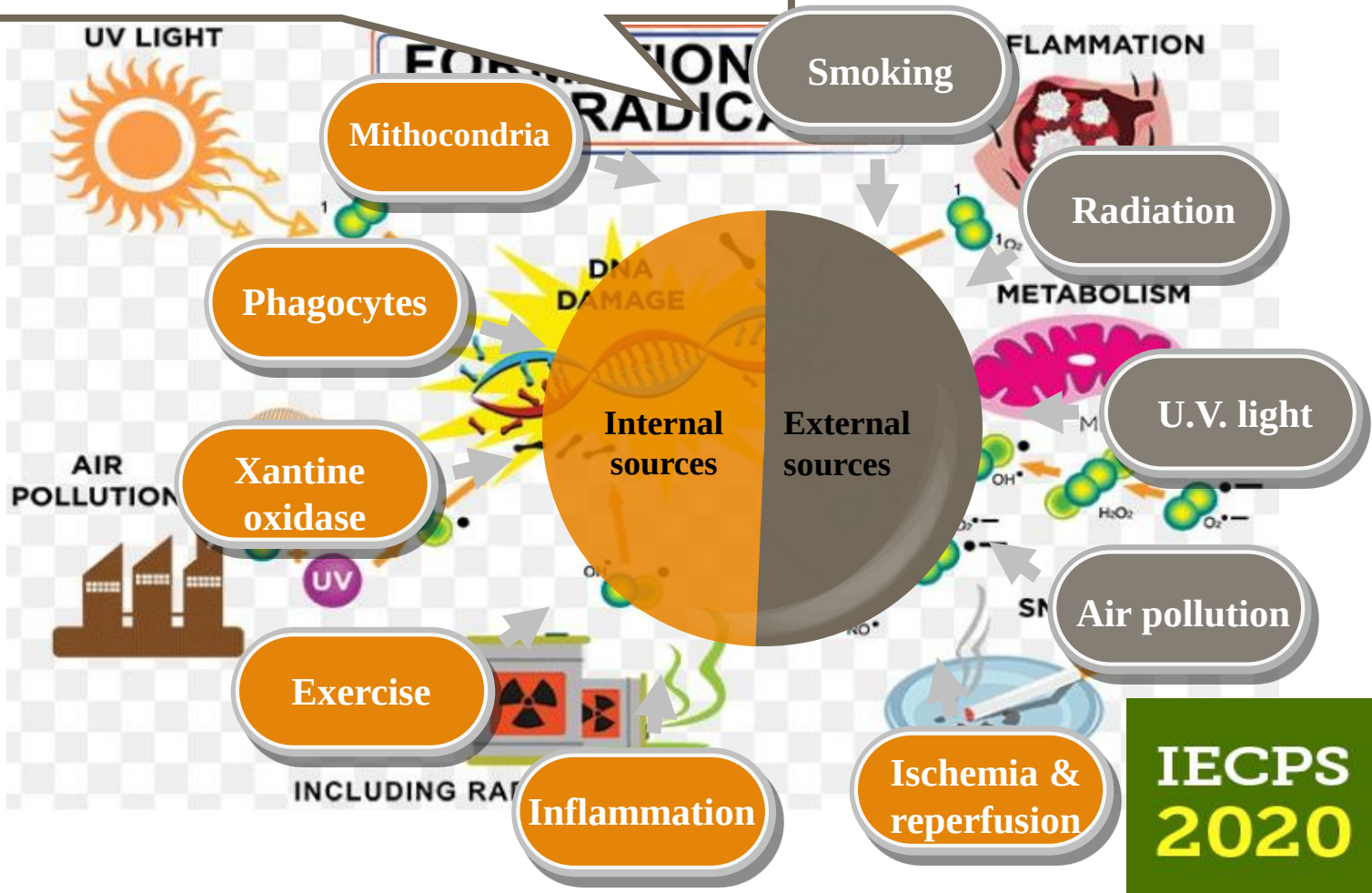
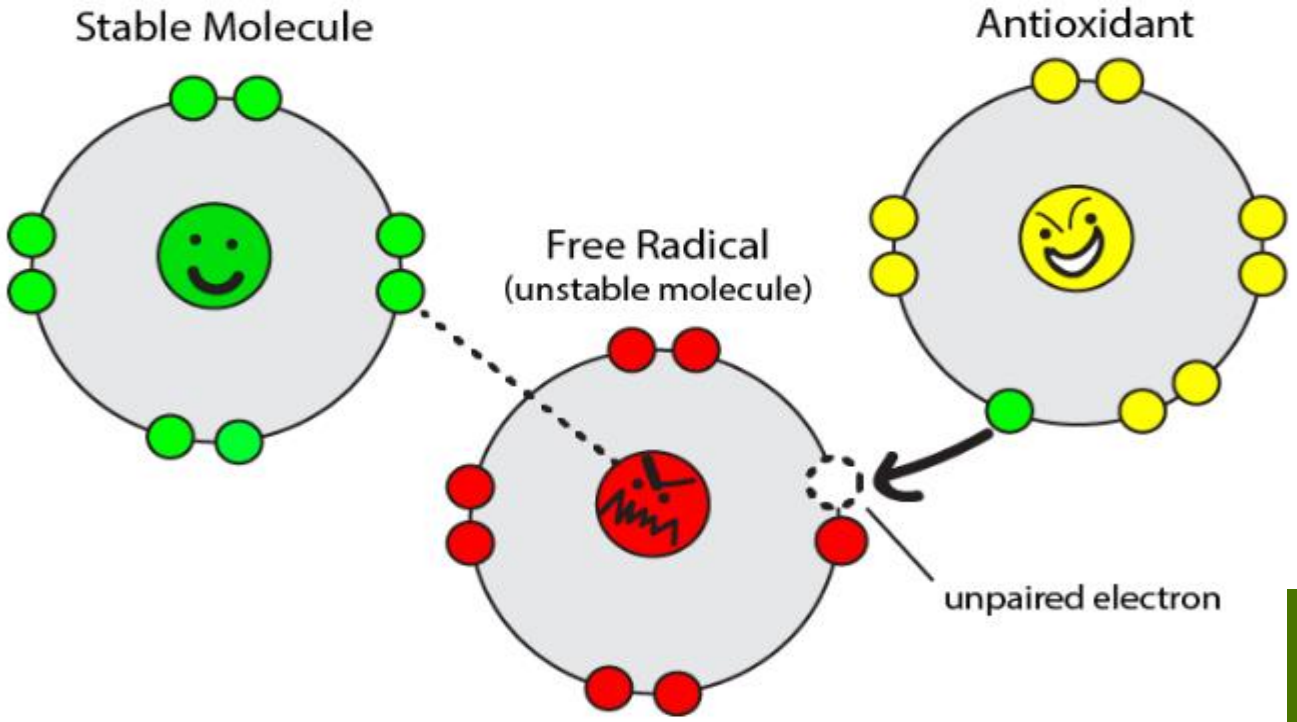
Source of free radicals

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.



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Selection cretiria of antioxidants

- 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

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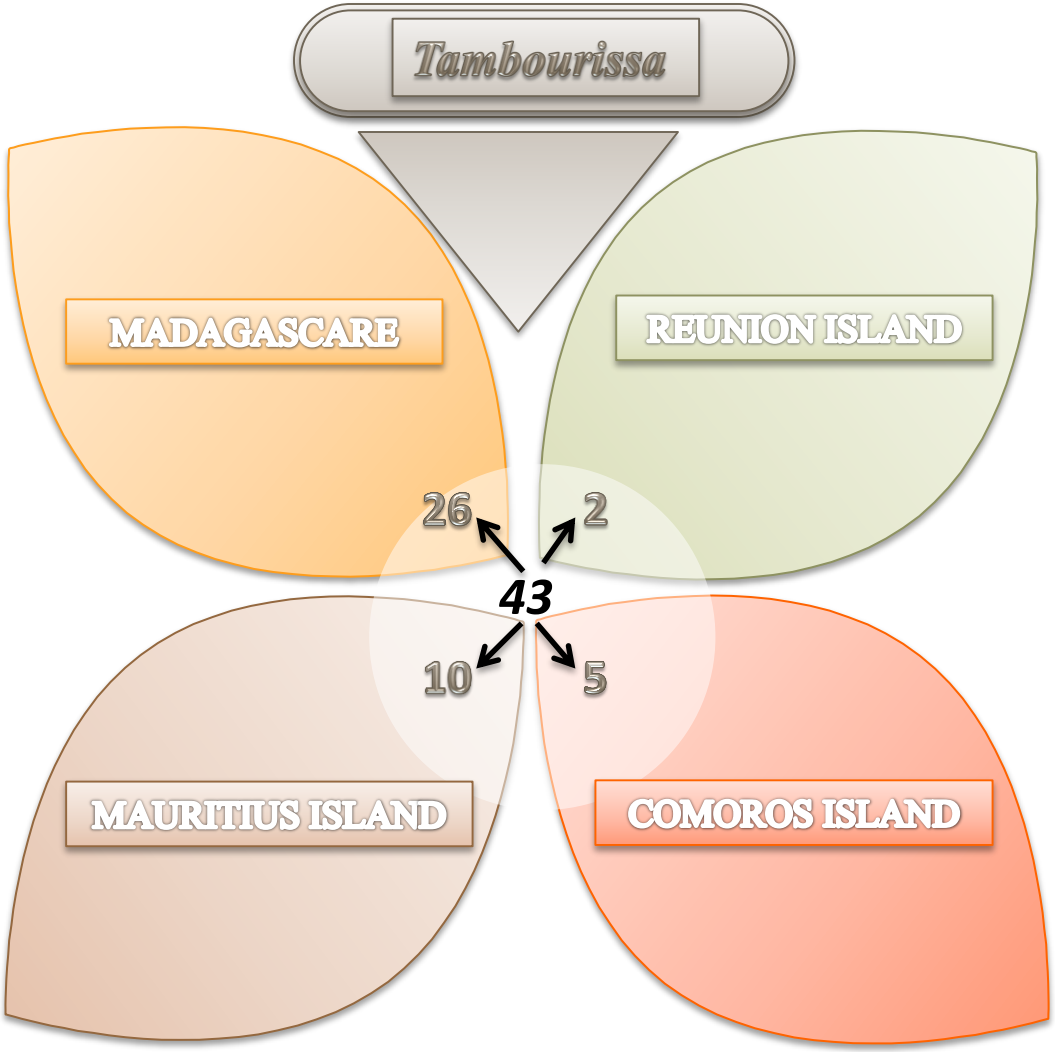
Natural compounds derived from medicinal plants play a central part in the health care and drug development in classical as well as advanced systems of medicine.

MEDICINAL PLANTS

- Easy availability
- negligible side effects
- Cost effectiveness


Tambourissa
MADAGASCARE
REUNION ISLAND
26
2
43
10
5
MAURITIUS ISLAND
COMOROS ISLAND

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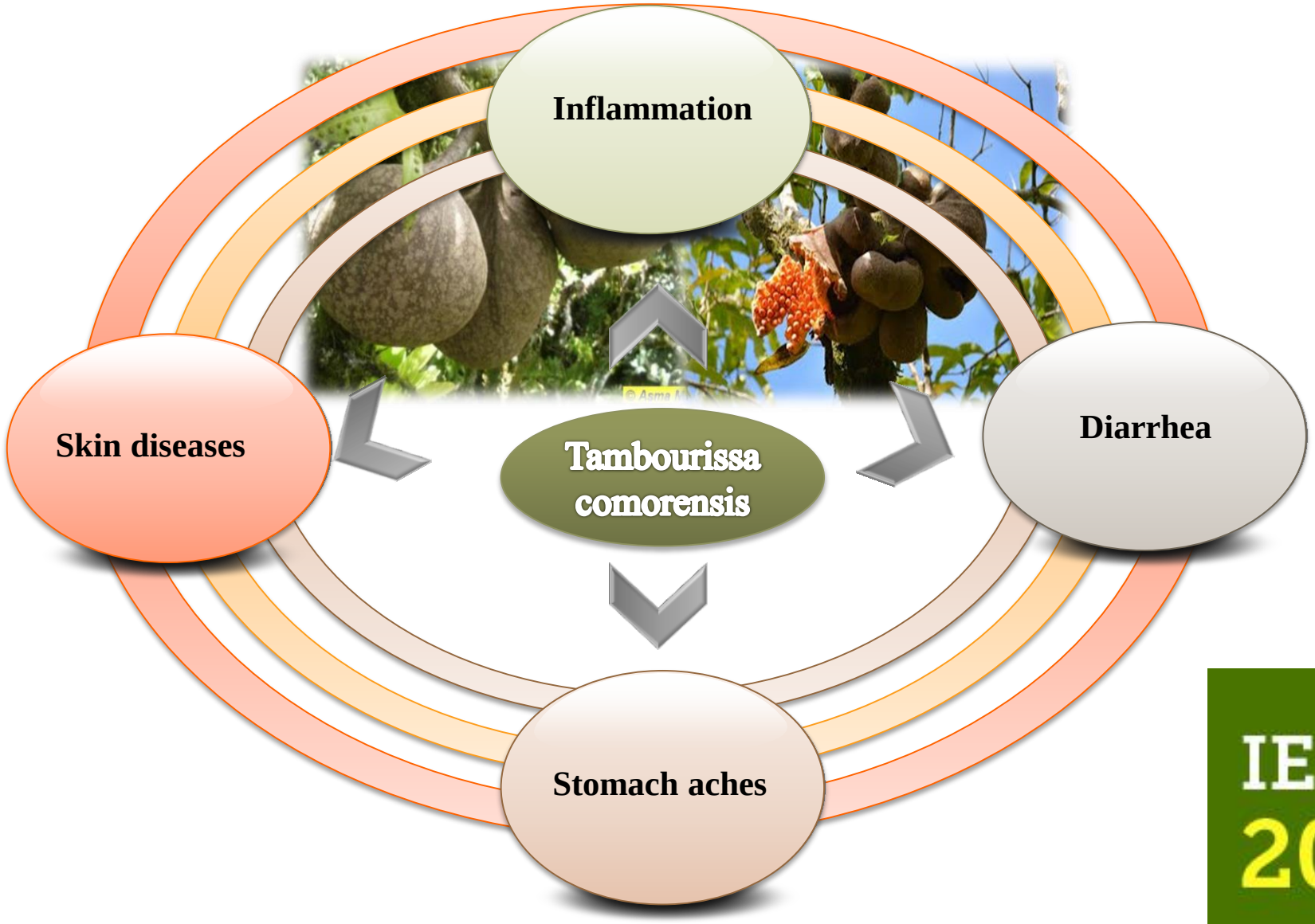
Inflammation
Skin diseases
Tambourissa comorensis
Diarrhea
Stomach aches

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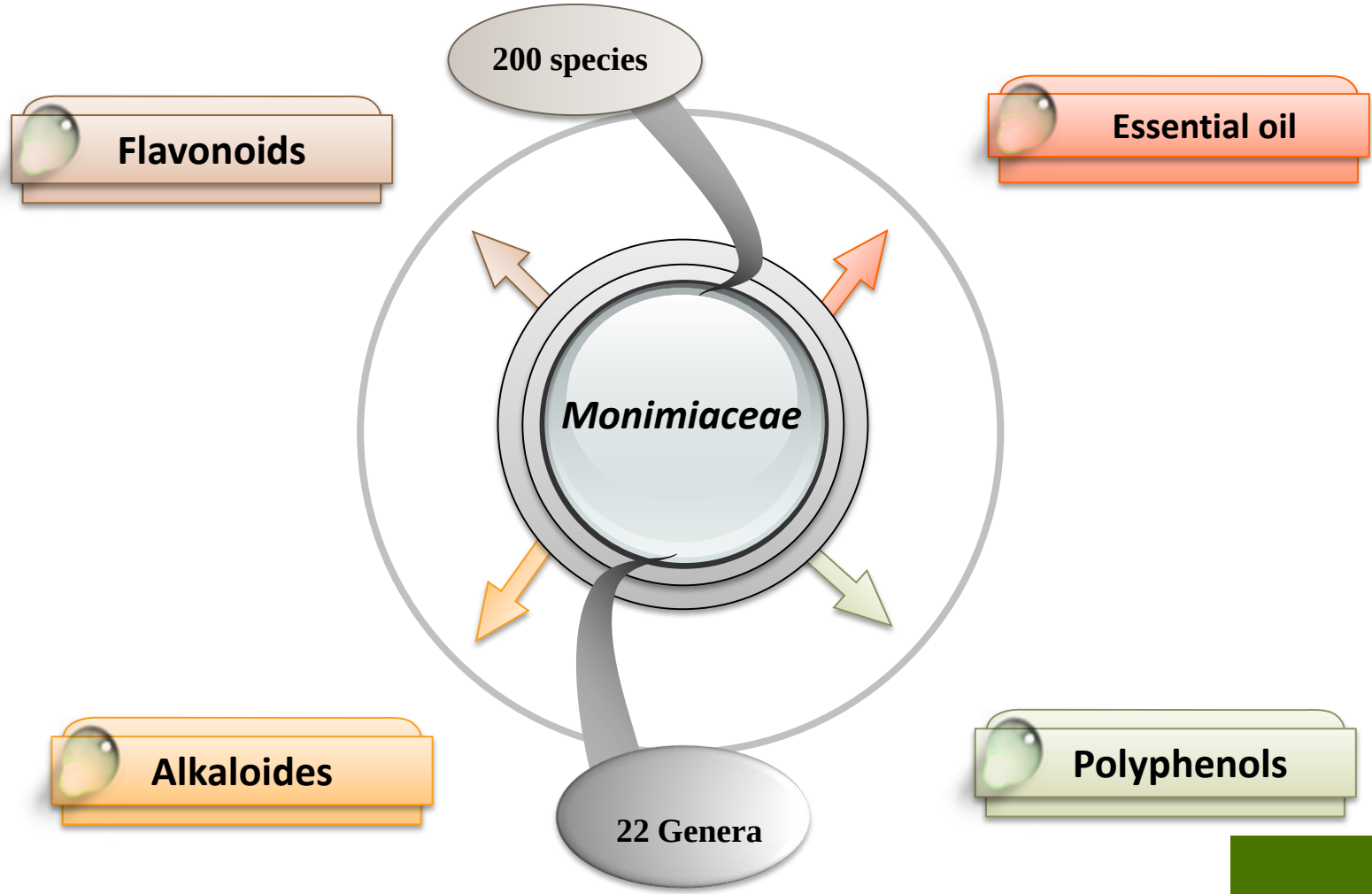

200 species
Flavonoids
Essential oil
Monimiaceae
Alkaloides
22 Genera
Polyphenols

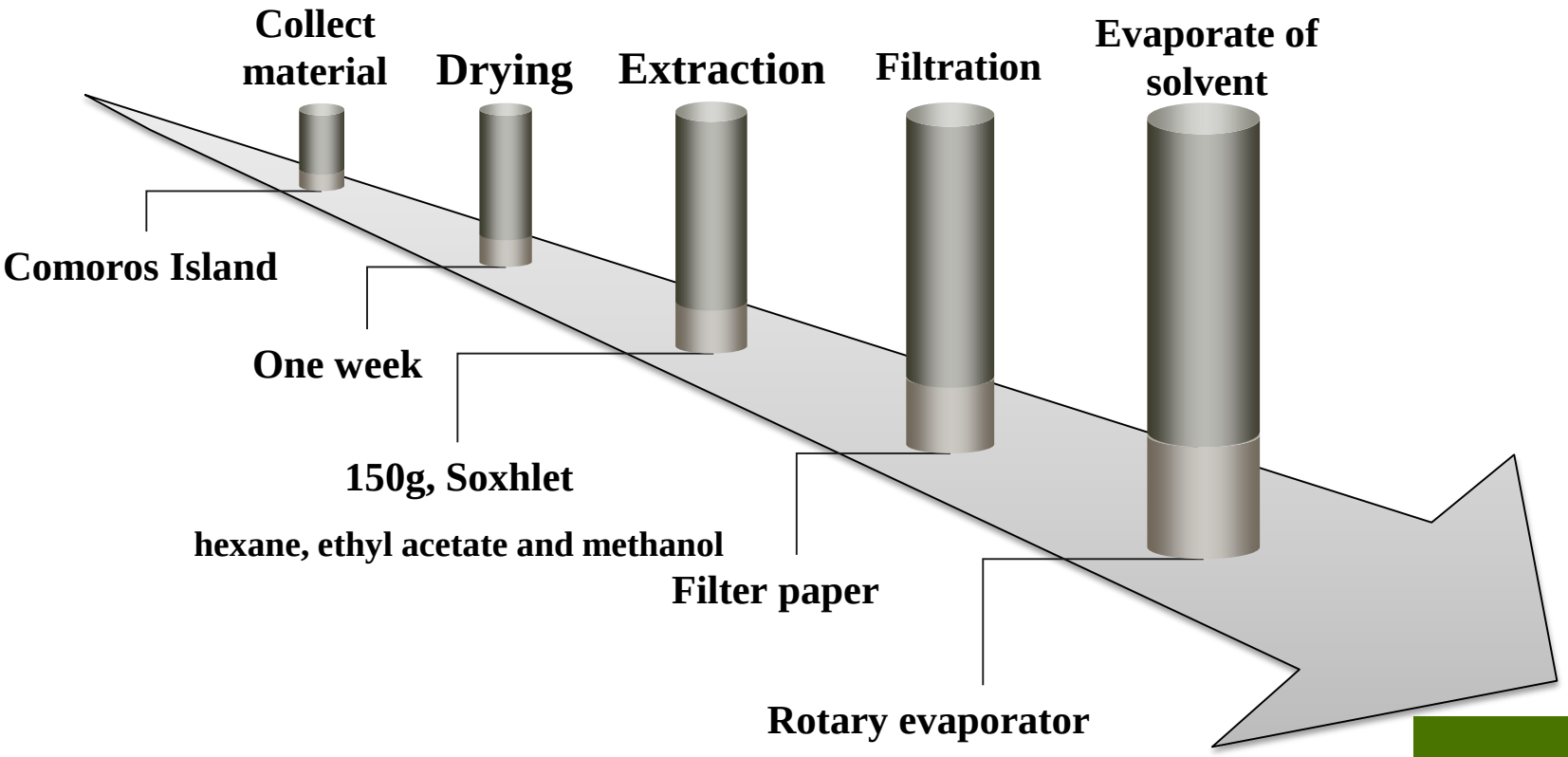
Aims and Objectives

The present investigation is undertaken by utilizing the plant *Tambourissa comorensis* with following objectives.

- **Extraction of the fruits 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



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Material and methods

2- PHYTOCHEMICAL ANALYSIS

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

Material and methods

3-TOTAL FLAVONOIDS CONTENT

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

Material and methods

TOTAL PHENOLIC CONTENT

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

Material and methods

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.

Material and methods

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

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 and** A_1: **absorbance of test compounds**

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

Material and methods

Reducing power scavenging activity (FRAP)

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 $FeCl_3$ (1%)

↓

Absorbance at 700nm

Ascorbic acid : standard

Material and methods

Total antioxidant capacity.

- 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).

Results

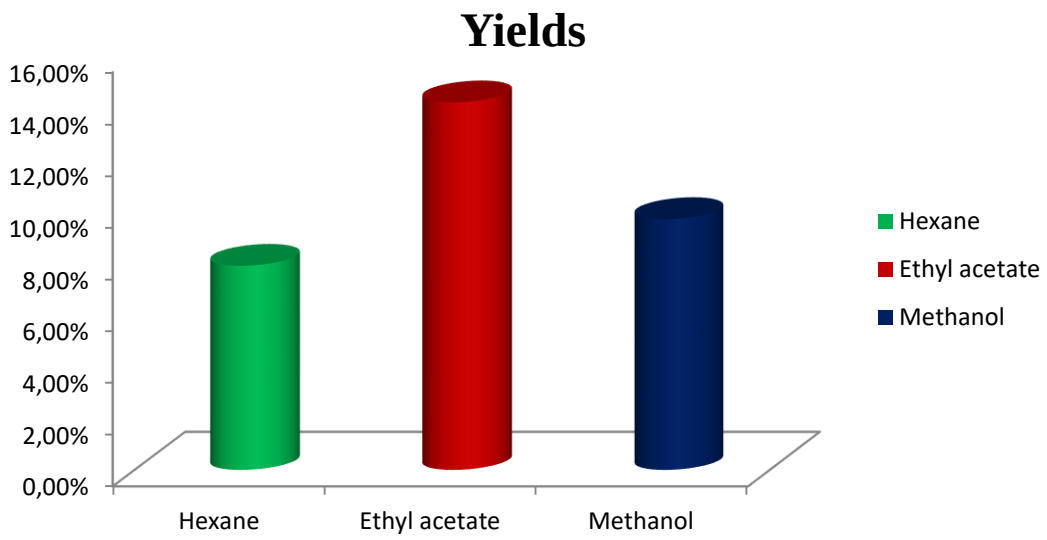


Figure 1*. Yields of hexane, ethyl acetate and methanol extracts of *T.comorensis

Results

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

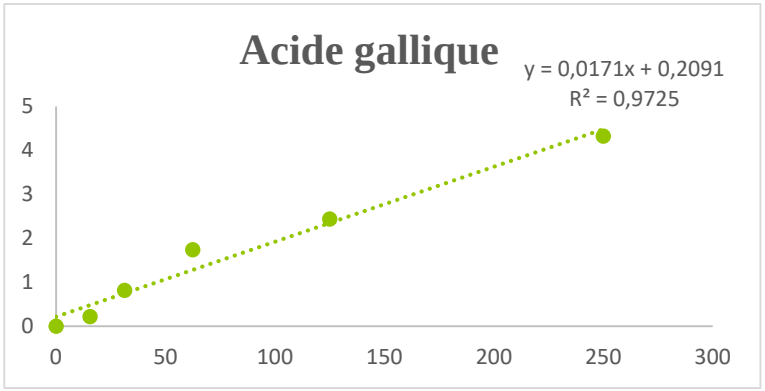
Table. 1 : Results of preliminary phytochemical screening of *T.comorensis* fruit extracts.

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 = 33.35 µg/mL GAE ; Methanol extract = 59.01 µg/mL GAE



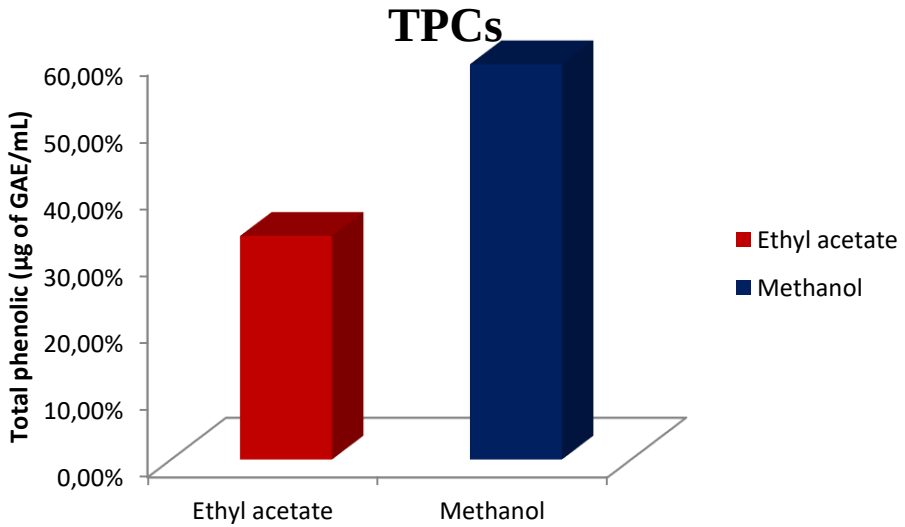


Fig 2. Total phenolics contents of ethyl acetate and methanol extracts of *T.comorensis*

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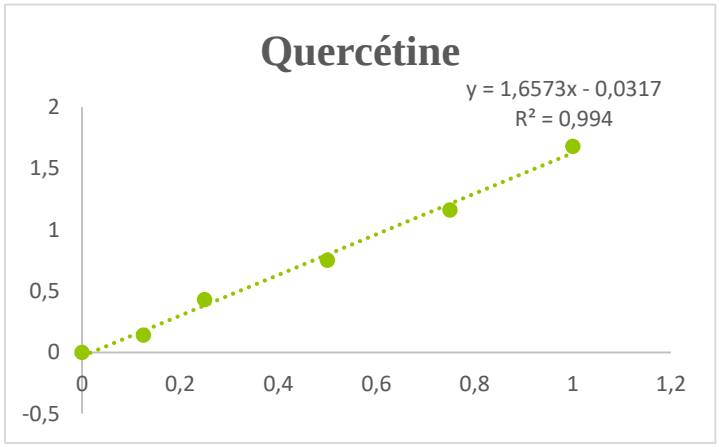
Results

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; R²=0.994.

Ethyl acetate extract = 0.08 mg/ml QE ; Methanol extract = 0.17 mg/ml QE



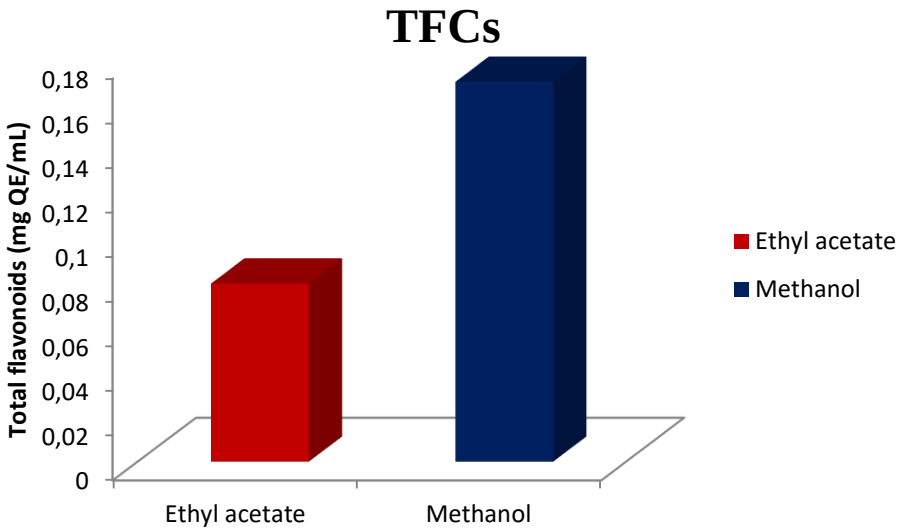


Fig 3. Total Flavonoid contents of ethyl acetate and methanol extracts of *T.comorensis*

Results

DPPH ANTIOXIDANT ACTIVITY

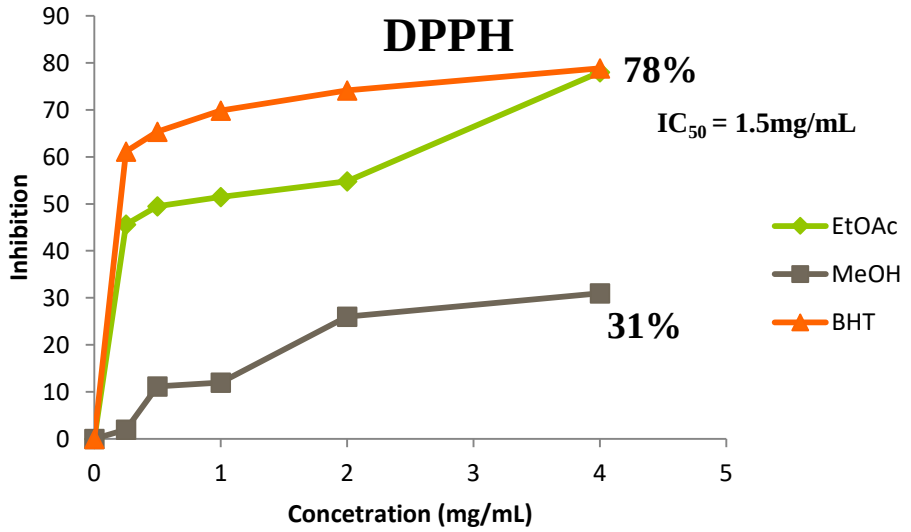


Fig 4. Antioxidant activity of *T.comorensis* fruit extracts against DPPH

None of samples evaluated showed a strong activity than BHT (IC_{50} = 0.5mg/mL)

Results

FERRIC REDUCING ANTIOXIDANT POWER

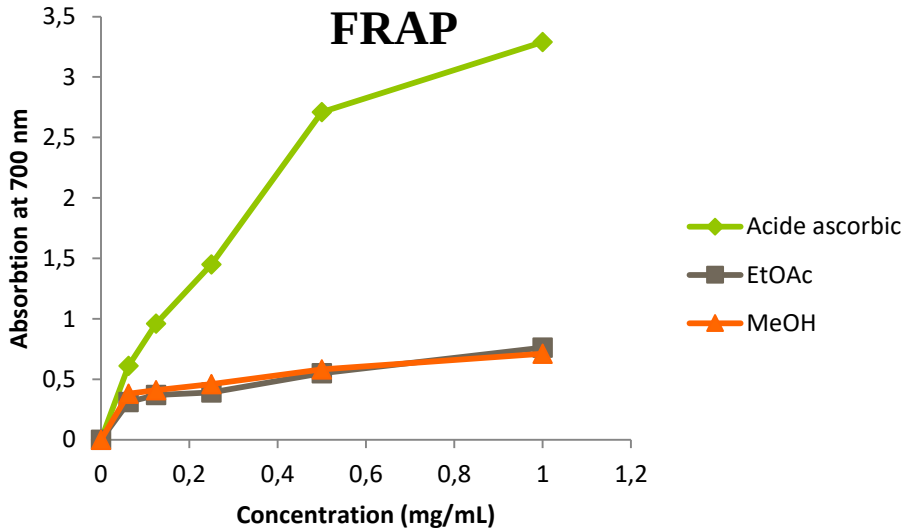


Fig 5. Reducing power of of *Tambourissa comorensis* fruit extracts

Results

TOTAL ANTIOXIDANT ACTIVITY

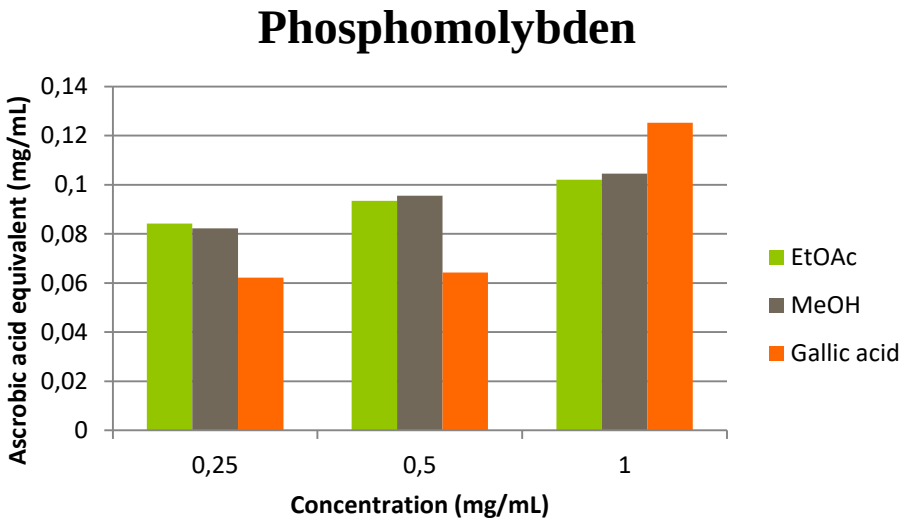


Fig 6. Total antioxidant capacity of *T.comorensis* fruit extracts

Conclusion

- Phytochemical screening demonstrates the presence of various classes of bioactive chemical constituents in all extracts of *T. comorensis* including sterols/steroids, terpenes /terpenoids, polyphenols, tannins and flavonoids.
- Total phenolic and flavonoid contents results showed a large dominance in a methanol extract.
- *T.comorensis* fruit extracts showed a significant antioxidant activity as well as, in low concetrations ethyl acetate and methanol extracts exhibited greater antioxidant than gallic acid as measured by total antioxidant capacity.
- These results suggested that the fruit of *T.comorensis* 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