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Phytochemical Screening and Evaluation of the Anti-Radical and Antimicrobial Activities of Extracts of *Artemisia annua* L. and *Artemisia afra* Jacq. Cultivated in Gabon

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Abstract:- ***Artemisia afra*** **and** ***Artemisia annua*** **are two medicinal plants used in the treatment of several diseases including malaria in South Africa and China respectively. In Gabon, these plant species are cultivated by the Maison de l'Artemisia, which promotes them in the fight against malaria. The objective of this work was to carry out a phytochemical screening and to evaluate the anti-radical and antibacterial properties of extracts of *A. afra* and *A. annua* cultivated in Gabon. Phytochemical constituents were identified using the tube reaction method. Bacterial susceptibility was determined using the diffusion method while scavenging activity was assessed by the DPPH free radical test. Phytochemical analysis showed that both plant extracts were rich in polyphenols and terpene compounds and possessed good anti-radical activity based on DPPH free radical scavenging. The aqueous extract of *A. afra* exhibited the strongest anti-radical activity with an IC_{50} of 0.02 mg/ml and an AAI of 2.4. The antibacterial activity shows that the ethanolic extract of *A. afra* inhibits the growth of *E. coli* while the ethanolic extract of *A. annua* inhibits the growth of *S. aureus* and *S. dysenteriae*. Our study shows that *A. afra* and *A. annua* plants grown in Gabon contain various families of chemical compounds and possess anti-radical and antibacterial activities. It also shows that *A. afra* has an anti-radical property superior to that of *A. annua*.**

***Keywords*:-** *Artemisia afra, Artemisia annua, Phytochemical Study, Anti-Radical Activity, Antibacterial Activity.*

I. INTRODUCTION

Reactive oxygen species exert a beneficial effect on cellular responses or immune function at low or moderate concentrations. However, at high levels, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cellular structures, including lipids, proteins and DNA. To protect itself from the deleterious effects of oxidative stress, the human body has a set of mechanisms such as the production of antioxidants which are produced naturally in situ or provided by food and/or as supplements. Antioxidants act as free radical scavengers by preventing or repairing damage caused by reactive oxygen species [1], [2]. To this end, several sources of plant-based antioxidants have been studied in recent years. Which therefore shows that the plant material contains many kinds of antioxidants with various activities [3]–[5].

Furthermore, infectious diseases of microbial origin are caused by the development of bacteria or fungi, some of which are pathogenic. Hence the importance given to the study of microbial diseases following the appearance of strains resistant to the most commonly used remedies [6]. In addition, microbial resistance to a wide range of infectious agents poses a growing threat to public health [2]. Thus, the abusive use of antibiotics favors the increase of bacterial resistance.

Indeed, inflammation which is a defense process of the body in response to the attack of a pathogenic agent can be considered as a source of reactive oxygen species through the activation of phagocytic cells during the phagocytosis. Also, inflammation accelerates the production of reactive oxygen species and decreases the oxidative defense capacity by favoring the appearance of oxidative stress, an important factor in the development of many diseases [6].

Thus, the use of medicinal plants with active biomolecules can be effective in improving treatment methods and possibly as a substitute for conventional therapies [7].

Artemisia afra Jacq., is one of the most popular and commonly used plants in traditional medicine in southern

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Africa. Due to its richness in secondary metabolites, *A. afra* has several biological activities including antimalarial, antioxidant and antibacterial activities [8]. *Artemisia annua* Linné, is an aromatic annual herb widely distributed in Asia, Europe and North America and used in the treatment of malaria and other diseases. Apart from its antimalarial activity, *A. annua* also shows anti-inflammatory, antipyretic and anti-cancer activities [9]. In vitro studies carried out on this plant also show that it has antibacterial and antioxidant properties [10], [9], [11].

It is in this context that the present work falls, the aim of which is to carry out a phytochemical screening in order to identify the different phytochemical constituents and to evaluate the anti-radical and antibacterial properties of the plant extracts of *A. afra*. Jack. and *A. annua* Linné cultivated in Gabon.

II. MATERIAL AND METHODS

➢ *Chemical Reagents and Medium*

Dimethyl sulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH), were obtained from FlukaChemika (Switzerland). Ethanol, hydrochloric acid, sulfuric acid, ammonia, sodium hydroxide, ferric chloride and other chemical reagents were from Sigma Chemical (Sigma-Aldrich Co., St. Louis, MO, USA) and analytical grade.

➢ *Plant Material*

The leaves and stems of *Artemisia annua* and *Artemisia afra* were collected in October 2021 at the Maison de l'Artemisia in the province of Estuaire, in the north-west of Gabon. The plant material had been previously dried at room temperature and away from the sun in the laboratory and ground into a fine powder. *A. annua* and *A. afra* powders were used for extractions by the maceration method. Briefly, 37 g and 39 g of *A. afra* and *A. annua* powder respectively were successively mixed with 200 mL of solvent (ethanol and water). After 3 h of extraction with each solvent, the extracts obtained were filtered on Whatman No. 1 filter paper. The ethanolic extracts were concentrated under reduced pressure with a BUCHI R-210 rotary evaporator at 60°C. As for the aqueous extracts, they were concentrated and freeze-dried using a freeze-dryer (Alpha 1-2 LDplus, Germany). The extracts obtained were stored at 4° C. until their use for the various tests.

➢ *Phytochemical Screening*

Phytochemical screening was carried out on the powder samples after extraction with the solvents water and ethanol using standard methods based on the coloring and precipitation reactions described by [12], [13] (table 1).

Table 1 Qualitative Analysis and Observations of Phytochemicals

Phytochemicals	Test	Observations (Positive results)
Total sugars	Molish's reagent	Violet staining at interphase
Reducing sugars	Fehling's reagent	Precipitated brick red
Alkaloids	Mayer's reagent Dragendorff's reagent	Yellowish-white precipitate Orange-red precipitate
Tannins	Ferric chloride	Blackish blue coloring
Sterols or Triterpenes	Liebermann-Burchard	Green, blue or purple coloring
Flavonoids	Cyanidin reaction with magnesium chip	Orange-pink, purplish-pink, cherry-red coloring
Carotenoids	Concentrated sulfuric acid	Red blue coloring
Coumarins	20% ammonia, UV reading at 360 nm	fluorescence (yellow, blue, blue-green, orange, purplish pink)
Quinones	Bornstraegen's reaction	Red or purple coloring
Saponins	Foam index test	Significant presence of foam of at least 1 cm
Monosaccharides and holosides	Sulfuric acid, heating in a water bath at 100°C	Red precipitate

➢ *Anti-Radical Activity*

The method described by Scherer and Godoy based on the DPPH free radical test, was used to evaluate the anti-radical activity index (AAI) [14]. A DPPH solution was prepared by dissolving 15 mg of DPPH powder in 150 ml of ethanol. Graduated concentrations of extracts from 0.0009766 to 0.5 mg/ml obtained by double dilution were prepared and 1 ml of each extract solution was mixed with 1 ml of ethanolic solution of DPPH. After a 30 min incubation period in a shaded temperature range, the absorbance was read at 517 nm. Ascorbic acid (Vitamin C) was used as a reference. The DPPH free radical inhibition (% I) was calculated as follows:

$$\% I = [(OD_{control} - OD_{sample}) / OD_{control}] \times 100$$

Where ODcontrol = Absorbance of control,
ODsample = Absorbance of sample

OD: Absorbance at 517 nm. The IC_{50} of the extracts and of the standard was calculated using the regression curves of the concentrations of extracts in (mg/ml) as a function of the percentage inhibition (% I) of the free radical DPPH.

- *The AAI was Calculated as follows:*

AAI = [DPPH] final (mg/ml) / IC_{50} (mg/ml)

The Scherer and Godoy criterion [14] was considered according to whether the extract of plants shows weak anti-radical activity when AAI < 0.5, moderate anti-radical activity when AAI is between 0.5 and 1.0, strong anti-radical activity when AAI is between 1.0 and 2 and very strong when AAI > 2.

➢ *Antimicrobial Activity*

- *Bacteria Tested*

The bacteria used in our study were four reference bacterial strains (*Escherichia coli* CIP 105182, *Shigella dysenteria* CIP 5451, *Staphylococcus aureus* ATCC 25293BHI and *Enterococcus faecalis* CIP 10397). These bacterial strains were obtained from the microbiota of the Laboratory of Research in Biochemistry (LAREBIO).

- *Preparation of the Bacterial Inoculum :*

Prior to any test, the bacterial strains were subcultured on a MULLER Hinton II (MH II) culture medium at 37° C. for 18 h so that they were in the exponential growth phase. The mature colonies were then removed and introduced into tubes containing physiological medium at 0.9% NaCl and homogenized until a turbidity equivalent to the 0.5 McFerland standard was obtained.

- *Disc Streaming Method* **:**

The in vitro antibacterial activity was evaluated by the diffusion method on MULLER Hinton II medium. The MH II plates were prepared by pouring the liquid medium into sterile Petri dishes (90 mm). The boxes were then left to cool and solidify.

After a control test carried out for 24 hours, the Petri dishes were inoculated by flooding with 2 ml of bacterial inoculum. The surplus of the bacterial suspension was removed from the petrie dish. The various extracts at 20 µl were loaded onto blotting paper discs 6 mm in diameter using a micropipette. The blotting paper discs loaded with extracts were placed on the surface of the solid medium. The extracts were left for diffusion for 5 min then the Petri dishes were incubated at 37° C. in an oven for 18-24 h. The negative control was prepared using extract dilution solvent (DMSO) and ciprofloxacin was used as a positive control. After 24 h of incubation, the clear zones of inhibition formed around the discs of blotting paper were measured using a ruler. The tests were carried out in triplicate.

III. RESULTS

➢ *Phytochemical Screening*

The phytochemical screening using qualitative analysis on the aqueous and ethanolic extracts of *A. afra* and *A. annua* shows the presence of the following chemical families: reducing sugars and coumarins in the aqueous and ethanolic extracts of the two plants while the tannins, sterols and triterpenes, oses and holosides are present in the ethanolic extract of the two plants. Quinones are present only in the ethanolic extract of *A. annua* (Table 2).

Table 2 Results of the preliminary phytochemical screening +++ : very abundant ; ++ : abundant ; + : not abundant ; - : not detected

Chemical families	**Extracts of *A. afra***		**Extracts of *A. annua***	
	Ethanol	**Water**	**Ethanol**	**Water**
Total sugars	-	-	-	-
Reducing sugars	+++	+++	+	+
Alkaloids	-	-	+	+
Tannins	+++	-	++	-
Sterols and Triterpenes	+++	-	+++	-
Flavonoids	-	-	-	-
Carotenoids	-	-	-	-
Coumarins	++	++	++	++
Quinones	-	-	+++	-
Saponins	-	-	-	-
Monosaccharides and holosides	++	-	+	-

➢ *Anti-Radical Activity*

The anti-radical activity of the extracts of *A. afra* and *A. annua* was evaluated by the DPPH method. The results obtained are represented in the form of straight line equations and the anti-radical activity of each extract is expressed as IC_{50} and AAI values (Table 3).

Table 3 Anti-Radical Activity of *A. afra* and *A. annua* Extracts by DPPH Free Radical Scavenging Method

Species		**Regression curve equation**	**R^2**	**IC_{50} (mg/ml)**	**AAI**
Artemisia afra	aqueous extract	Y = 353.34X + 42.65	0.97	0.02	2.4
	Ethanol extract	Y = 254.19X + 7.79	0.96	0.17	0.3
Artemisia annua	aqueous extract	Y = 87.979X + 42.21	0.96	0.089	0.56
	Ethanol extract	Y = 230.68X + 4.99	0.96	0.2	0.26
Control	Vitamin C	Y = 6.76X + 2.03	0.985	0.00712	7.02

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In comparison with the other extracts, the aqueous extract of *A. afra* has the greatest anti-radical activity marked by a strong inhibition of the free radical DPPH (IC_{50} = 0.02 mg/ml, AAI = 2.4).

However, compared to the anti-radical activity of vitamin C (IC_{50} = 0.00712 mg/ml, AAI = 7.02), it is observed that the anti-radical activity of the aqueous extract of *A. afra* is very weak.

➢ *Antibacterial Activity*

The antibacterial activity of the aqueous and ethanolic extracts of *A. afra* and *A. annua* was evaluated on a few bacterial strains using the method of diffusion on agar medium. The results of the antibacterial activity are shown in Tables 3 and 4. The results show that the ethanolic extract of *A. annua* produced a wide zone of inhibition on the bacterial strains such as *S. aureus* ATCC 25293BMI and *S. dysenteriae* CIP5451. The ethanolic extract of *A. afra* also showed a large zone of inhibition on the bacterial strain *E. coli* CIP105182. In general, the antibacterial activity demonstrated by the extracts was much weaker than that of the reference antibiotic (Table 4).

Table 4 Antibacterial Activity of *A. afra* and *A. annua* Extracts

Bacterial strains	**Inhibition zone diameter (mm)**					
	Artemisia afra		***Artemisia annua***		**Control**	
	Ethanol	Water	Ethanol	Water	DMSO	Ciprofloxacin
E.coli	-	19±1.9	-	-	-	24±2.4
S. aureus	7.67 ± 0.7	7.33 ± 0.75	10.33 ± 1.05	7.67 ± 0.8	-	36±3.35
E.faecalis	9±0.9	-	7.33 ± 0.75	7.67 ± 0.75	-	38.33±3.85
S. dysenteria	-	-	16.33±2.4	-	-	42±4.15

IV. DISCUSSION

➢ *Artemisia Afra Jacq.*

Screening of *A. afra* extracts reveals the presence of phytochemical constituents which can have anti-radical and antibacterial properties including tannins, triterpenes and coumarins. The presence of reducing sugars and coumarins was detected in the two extracts while the presence of tannins, sterols, triterpenes, oses and holosides was detected in the ethanolic extract. These results differ from the results obtained by Sunmonu and Afolayan, who during their study detected a large amount of phenolic compounds including phenols, flavonoids, flavonols and pro-anthocyanidins for the aqueous extract [15]. The observed difference may be due to the geographical variation which could induce a variation in the composition of secondary metabolites of the plant [16], [17].

To this we can also add the duration of the extraction which was quite short. The results obtained show that the aqueous extract of *A. afra* has an anti-free radical capacity superior to that of the ethanolic extract, which could be explained by the structure or the class of compounds present in our extracts. The results obtained in this study correlate with the results obtained by Sunmonu and Afolayan, demonstrating that the aqueous extract of *A. afra* possesses a significant anti-radical capacity [15].

Furthermore, the results obtained by More et al., [18] demonstrated that the ethanolic extract of *A. afra* has a good anti-radical capacity with an IC_{50} value of 22 µg/ml. This result differs from that obtained in our study for the ethanolic extract (0.17 mg/ml) but similar to that obtained with the aqueous extract (0.02 mg/ml).

Concerning the antibacterial activity, the results obtained in this study show that the aqueous and ethanolic extracts of *A. afra* inhibit bacterial growth. These results also show that the extracts of *A. afra* have an effect on strains *S. aureus, E. faecalis and E. coli*. This is in agreement with the results obtained by Muleya et al., [19] who demonstrated that the extracts of *A. afra* possessed bacterial activity against *E. coli*, *S. aureus* and *E. faecalis*.

➢ *Artemisia annua Linnaeus.*

The results obtained from the phytochemical screening carried out on the extracts of *A. annua* revealed the presence of tannins, sterols, triterpenes, oses and holosides in the ethanolic extract, while the presence of reducing sugars and coumarins revealed was detected in the aqueous and ethanolic extracts. The results obtained from the phytochemical screening do not show any conformity with the results obtained for the aqueous extract by Us et al., [20] which reveal the presence of steroids, terpenoids, alkaloids, flavonoids, glycosides, saponins, tannins , phenolic compounds and anthraquinones in the aqueous extracts after phytochemical analysis of the extracts. The same is true for the ethanolic extract insofar as our results differ from the results obtained by Massiha et al., [7] for whom the phytochemical screening of the ethanolic extract revealed the presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides, tannins, phenols, quinines, saponins, terpenoids and phlobatannins.

In this study, aqueous and ethanolic extracts of *A. annua* were found to possess free radical scavenging ability against the free radical DPPH. The results obtained reveal a moderate anti-radical capacity for the aqueous extract but weak for the ethanolic extract. These results are in agreement with the results obtained by Kim et al., [21] who demonstrated that the aqueous extract of *A. annua* had the greatest anti-radical capacity (91.0%) compared to the extracts of ethanol, methanol and acetone.

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Previous studies have shown that the essential oil as well as the extracts of *A. annua* have antibacterial activity on various bacterial strains such as *Staphylococcus aureus*, *Escherichi coli*, *Enterococcus faecalis*, *Enterococcus hirae*, *Candida albican*, *Candida krusei*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Bacillus subtilis* [22], [9], [7]. However in this study the antibacterial activity of the plant was evaluated on four bacterial strains. And the results obtained show that the extracts of *A. annua* inhibit the growth of *S. aureus*, *E. faecalis* and *S. dysenteriae* for the ethanolic extract, *S. aureus* and *E. faecalis* for the aqueous extract. A recent study by Massiha et al., [7] showed that extracts of *A. annua* inhibit the growth of bacteria tested including *S. aureus*, *E. coli* and *E. faecalis* at different concentrations. This corroborates the antibacterial effect of *A. annua* extracts observed on *S. aureus* and *E. faecalis* except *E. coli* in this study. According to Gupta et al., [10] *A. annua* has an antibacterial effect on *E. coli* and *S. aureus*. Another study conducted by Tejehmiri et al, [23] demonstrated that the ethanolic extract of *A. annua* inhibits the growth of *S. aureus*, *E. coli* and *S. dysenteriae*.

V. CONCLUSION

Ultimately, our work focused on the phytochemical analysis, the anti-radical and antibacterial properties of extracts of *A. afra* and *A. annua* grown in Gabon. The results demonstrated the presence of various phytochemical constituents including polyphenols in the extracts of *A. afra* and *A. annua*. The aqueous and ethanolic extracts have been shown to possess anti-radical activities but below that of vitamin C. The extracts have also shown to have an inhibitory activity on the growth of some bacteria *including E. coli*, *S. aureus* and *S. dysenteriae*.

➢ *Abbreviations*

- *AAI : Anti-radical activity index;*
- *DPPH : 2,2-diphenyl-1-picryl-hydrazyl*

➢ *Conflicts of Interest*

The authors advise that there are no competing interests to declare. The authors alone are responsible for the content and writing of this article.

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