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Original Research Article

Aframomum subsericeum and *Aframomum alboviolaceum* leaf essential oils exhibit testosterone antagonistic and oestrogen synergic effects in male Wistar rats: potential candidates for prostate cancer treatment

Adjoffoin Chiara Nange¹, Sefirin Djiogue^{1*}, Ambamba Akamba Bruno Dupon²,
Bakam Yengwa Berlise¹, Kevine Silihe Kamga³, Njamen Dieudonne¹

¹Department of Animal Biology and Physiology, University of Yaoundé 1, P.O. Box 812 Yaoundé, Cameroon

²Department of Biochemistry, Faculty of Science, University of Yaoundé 1, P.O. Box 812 Yaoundé, Cameroon

³Department of Pharmacotoxicology, Faculty of Medicine and Biomedical Sciences, University of Yaounde 1, P.O. Box 1364 Yaounde, Cameroon

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*Correspondence:

Dr. Sefirin Djiogue,

Email: sefirin.djiogue@facsciences-uy1.cm

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ABSTRACT

Background: *Aframomum* species are routinely used as spices in many traditional Cameroonian meals due to their sweet fragrance. Many species of the *Aframomum* species are also used to treat cancer, pain, arthritis, and stomach disorders. This work was performed to determine the chemical composition, antiandrogenic, and estrogenic properties of *Aframomum subsericeum* and *Aframomum alboviolaceum* leaf essential oils.

Methods: The chemical components of *A. subsericeum* and *A. alboviolaceum* essential oils were identified by gas chromatography-mass spectroscopy. The anti-androgenic and estrogenic properties were investigated in vivo in physically and chemically castrated Wistar rats, respectively.

Results: Fifteen compounds versus nine compounds were identified in *A. subsericeum* and *A. alboviolaceum* essential oils, respectively. The main compounds of *Aframomum subsericeum* were cyclohexene, 1-methyl-4-(1-methylethylidene) (10.03%), γ -terpine (12.22%), and myrtenyl acetate (8.52%), while those of *Aframomum alboviolaceum* were γ -terpinene (20.07%), caryophyllene (11.58%), myrtenyl acetate (7.22%), and (1R)2, 6, 6 trimethylbicyclo [3.1.1] hept-2-ene (6.97%). The results of the anti-androgenic test reveal that both *A. subsericeum* and *A. alboviolaceum* induced a significant decrease in androgen-dependent organs, especially the prostate and seminal vesicle, at doses of 200 mg/kg and 100 mg/kg. Interestingly, *A. alboviolaceum* showed oestrogen synergic effects on the above organs.

Conclusions: So, this study reveals mainly the presence of diterpenoids in both *A. subsericeum* and *A. alboviolaceum*, which are potential candidates for prostate cancer treatment. The *in vivo* results show that *Aframomum* leaf essential has antiandrogenic and oestrogen synergic properties, suggesting that the oils could provide a safe natural drug for prostate cancer treatment.

Keywords: *Aframomum* species, Antiandrogenicity and oestrogenicity, Chemical composition, Essential oils

INTRODUCTION

The prostate gland is sensitive to steroid hormones, especially androgens and oestrogens, which influence its growth and development. These hormones also promote or retard the development of benign and malignant prostate

tumours as well.¹ The withdrawal of androgens by physical or chemical castration using antiandrogens has been a long-time treatment for prostate tumors.² Oestrogens have also been used in the treatment of prostate tumours by negative feedback mechanisms, interactions with beta receptors, and/or as co-treatment to manage androgen deprivation symptoms like osteoporosis.³⁻⁵

Side effects are frequently linked to prostate cancer treatment, both short- and long-term, and are typically indicative of the drug's mode of action. For example, the use of goserelin and leuprolide in the treatment of prostate cancer lowers oestrogen levels and is considered the main cause of neurological disorders such as depression, anxiety, and memory loss, as well as osteoporosis, and elevated glucose levels.⁴ Exorbitant expenses, significant recidivism rates, and resistance phenomena further render prostate tumour management inadequate. Prostate illnesses and its complications are now treated worldwide with natural products and functional diets as alternatives or complements.

Aframomum is a family of rhizomal herbs with about 61 species in Africa and 20 species in Cameroon. They are known to produce essential oils in all their body parts. Like most species, the seeds and leaves of *Aframomum subsericeum* and *Aframomum alboviolaceum* are used as spices in Cameroon, but little study has been done on their biological activities. Most of the *Aframomum* species have shown antioxidant, anti-inflammatory, and cytotoxic activities in vitro and in vivo. Their essential oils are characterized by monoterpenes and diterpenes which are reference molecules in cancer treatment.⁹⁻¹² The main interest of this study was to determine the chemical composition and to investigate the antiandrogenic and estrogenic properties of *A. subsericeum* and *A. alboviolaceum* leaf essential oils as possible modes of action against prostate tumours.

METHODS

Plant materials

The leaves of *A. subsericeum* and *A. alboviolaceum* were collected in the west and central regions of Cameroon, respectively. They were then identified at the Cameroon National Herbarium (Yaoundé), where representative samples of the species are deposited under the reference numbers 37744/HNC (*A. subsericeum*) and 14758SFRcam (*A. subsericeum*). The material was dried in the laboratory under sheds, and leaf essential oils were obtained by hydrodistillation.

Chemical reagents

The standard drug, bilucamide (Casodex®), was obtained from Pierre Fabre (Boulogne, France). Enanthate testosterone (Androtardyl®) was purchased from Bayer Pharma AG (Berlin, Germany), oestradiol valerate (Progynova® 2 mg, laboratoire Delpharm, Lille, France), and fulvestrant (ICI 182 780) (Faslodex®) was purchased from Tocris Biosciences (Bristol, UK).

Animals

Healthy mature male Wistar rats were obtained from the breeding unit of the animal physiology laboratory, University of Yaoundé I. The rats were 45-60 days old,

weighing around 100-120 g (antiandrogenic test), and 60-75 days old, weighing approximately 120-140 gm (oestrogenic test). The rats were kept in groups of five in plastic cages with a natural light/dark cycle of approximately twelve hours and a standard laboratory temperature of twenty-five degrees Celsius. A typical soy-free rat diet of 50% maize, 5% bone flour, 10% wheat, 5% wheat bran, 14.0% fish flour, 8% groundnut paste, 7% crushed palm kernel and 1% vitamin complex was available to the animals without restriction. The Joint Institutional Review Board for Animal and Human Bioethics verified the conditions of animal housing and every experiment (Ref No: BTC-JIRB2023-078).

Phytochemical identification analysis

The *A. subsericeum* and *A. alboviolaceum* essential oil biologically active compounds were identified by gas chromatography-mass spectrometry (GC-MS) as previously described by Edewor et al.¹³ The essential oils were separately diluted in HPLC-grade hexane and filtrated using Whatman No. 1 paper. The instrument used was a mass selective detection (MSD) in inert scan mode Agilent 5975C with triple-axis detector (Agilent Technologies, Santa Clara, USA). An Agilent 19091S 433HP-5MS capillary column with dimensions of 30 m × 250 µm × 0.25 µm was integrated into the instrument. Helium gas was used as the carrier gas, with a flow rate of 0.84 ml/minute. The injection temperature was set at 300°C, while the oven temperature was initially set at 100°C and was gradually increased at a rate of 5°C/minute to 280°C. The run time was 36 minutes. One microliter of sample was introduced into the column. For the MS programme, the inlet line temperature was 150°C, the source temperature was 230°C, the ionization energy was 70 eV, and the mass scan (m/z) was between 50 and 600. The mass spectrometer was connected to a Chem Station G1701EA E.02.02.1431 mass spectra library management system (NIST 11), and the identification of oil constituents was based on their mass spectra.

Evaluation of the antiandrogenic activities of *A. subsericeum* and *A. alboviolaceum* in vivo

The experiment was conducted in compliance with 2009 OECD guideline 441.¹⁴ With the exception of the normal control group, which had a sham operation, all fifty-six-week-old Wistar rats were castrated after being acclimated to their new environment. The animals were divided into ten groups of five animals each, following a week of hormonal decline. A typical control group was given subcutaneous maize oil and the dilution material (0.1% tween) orally. The second group, known as the negative control, was given 0.1% oral and subcutaneous testosterone injections. The third group (positive control) was given subcutaneous testosterone injections (0.4 mg/kg/day) and oral antiandrogen casodex (3 mg/kg/day). The remaining six were given subcutaneous testosterone injections together with essential oils from *A. alboviolaceum* or *A. subsericeum* leaves at doses of 10,

100, and 200 mg/kg. Rats' treatment was done for ten days, after which they were put under mild anaesthesia and decapitated with 10 mg/kg of diazepam and 50 mg/kg of ketamine. The ventral prostate, seminal vesicles, Tyson glands, glands, and elevator muscles were removed, defatted, and weighed right away

Evaluation of the oestrogenic activity of *A. alboviolaceum* in vivo

The aim of this experiment was to assess whether Aframomum essential oils possess oestrogenic properties in male rats as a possible mechanism of action on prostate tumors. The experiment was conducted according to the modified protocol of Jasem and Tawfeek.¹⁵ To do this, 50 male rats were divided into 10 groups. The normal group received 0.1% tween, the second group received an oestrogen receptor blocker (Fulvestrant) at a dose of 300 µg/kg BW, the third group was treated with letrozole at a dose of 400 µg/kg, and the fourth group was treated with letrozole and estradiol valerate (E2V). The rest of the groups were cotreated with letrozole, E2V, and *A. alboviolaceum* essential oils at doses of 10, 100, and 200 mg/kg. Fulvestrant (ICI) and corn oil were administered by the SC pathway, while the rest of the substances were administered orally. The treatment lasted for 30 days, after which the animals were sacrificed and androgen-dependent organs (prostate, epididymis, seminal vesicles, testes, deferent canal, and penis) were removed and weighed.

Statistical analysis

The results are expressed as the mean $\pm$ standard error of the mean (SEM). The results were analyzed using a one-way ANOVA parametric test followed by Dunnett's multiple comparison test. The means of the different parameters obtained in the test groups were compared with the negative control (Testo), while the normal control (NOR) was compared with the negative control using a nonparametric student's t test. The differences between the groups were considered significant if the probability was $p < 0.05$.

RESULTS

Yields

The yields of two Aframomum leaf essential oils obtained by hydro distillation show that *A. alboviolaceum* (0.34%) produces the highest yields, about three times more than that of *A. subsericeum* (0.099%).

GC-MS characterization of *A. alboviolaceum* and *A. subsericeum* leaf essential oils

The chromatograms obtained from GC-MS analysis of the two-leaf essential oil (Figure 1) reveal the presence of 15 compounds in *A. subsericeum* and 8 compounds in *A. alboviolaceum*. The compound names, their retention time, and the percentage area under the curve are depicted in Table 1. Compounds with the highest percentage of occurrence are seen in Figure 2.

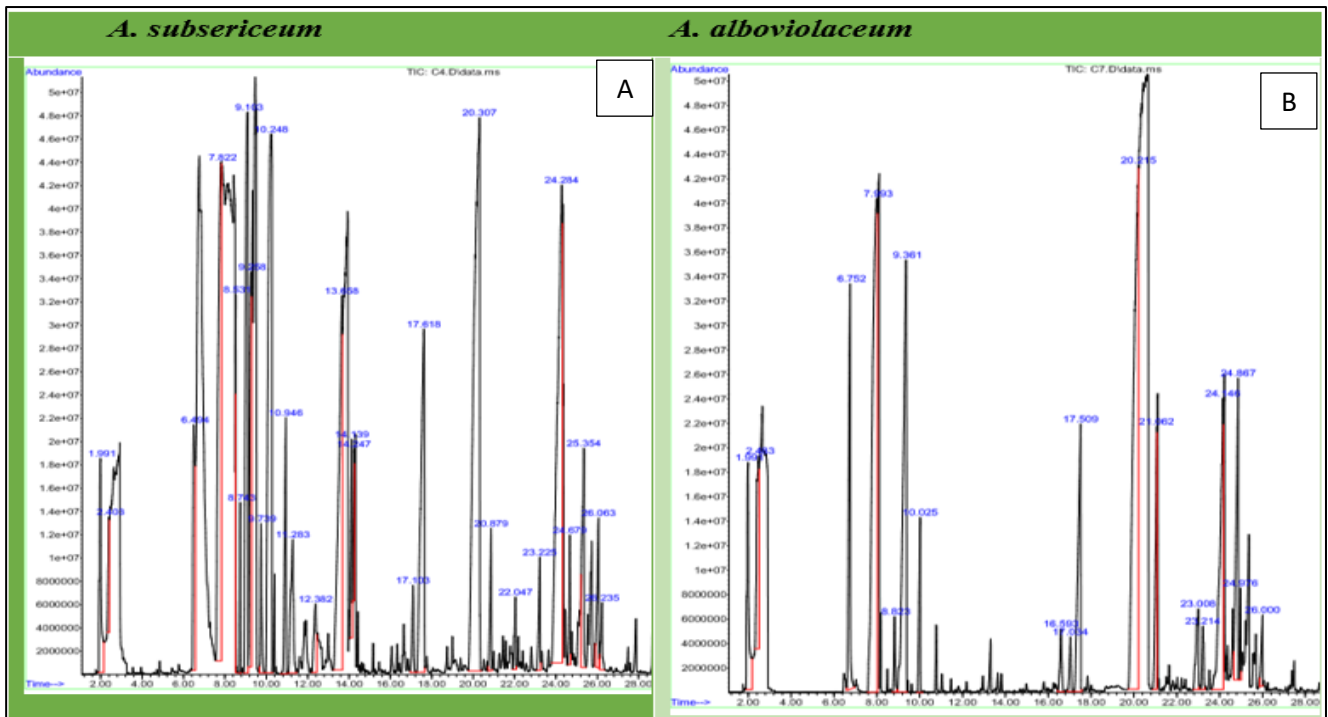


Figure 1 (A and B): Chromatogram of *A. subsericeum* and *A. alboviolaceum* leaf essential oils.

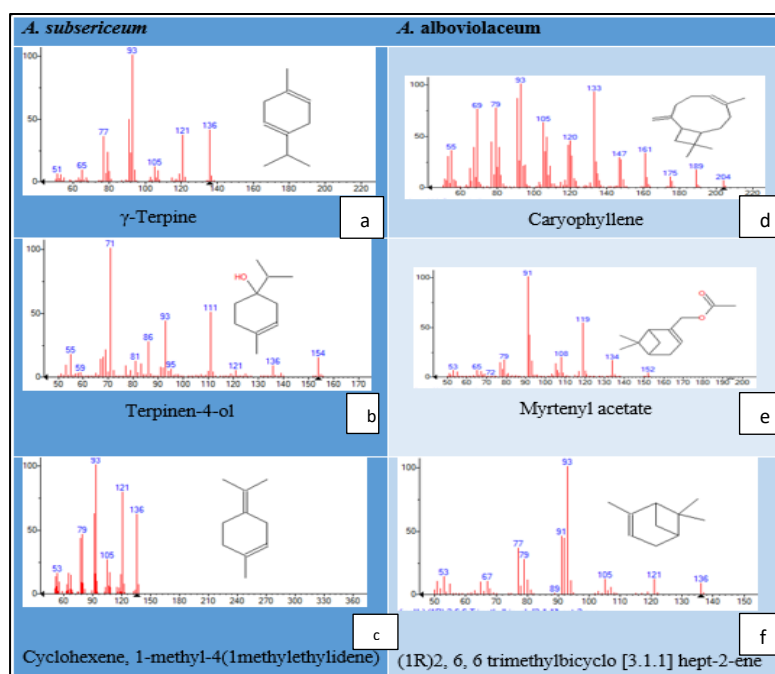


Figure 2 (a-f): Mass spectrum and structures of the most abundant compounds identify in *A. subsericeum* and *A. alboviolaceum*.

Table 1: Chemical composition of two Aframomum leaf essential oils.

Compound name	Retention time (minutes)		Area %	
	AC4	AC7	AC4	AC7
(1R)2, 6, 6 trimethylbicyclo [3.1.1] hept-2-ene	-	6.752	-	6.97
Bicyclo [3.1.1] heptane, 6,6 dimethyl-2-methylene	-	12.954	-	0.20
Bicyclo [3.1.0] 3-ol, 6,6 dimethyl-2-methylene	12.382	-	1.25	-
γ-Terpinene	-	7.976	-	20.07
Terpinen-4-ol	10.231	7.976	12.22	3.97
Alpha -Terpineol	13.658	-	10.01	-
Myrtenyl acetate	14.139	-	1.82	-
Caryophyllene	17.618	17.509	8.52	7.22
Caryophyllene oxide	-	20.055	-	11.58
Humulene	23.225	24.146	1.16	3.97
Bicyclo [3.1.1] he-2-ene, 4-methy-1 (methyletyl)	20.879	21.062	1.4	3.89
Beta -myrcene	6.494	-	3.97	-
Cyclohexene, 1-methyl-4-(1-methylethylidene)	8.743	-	0.8	-
Beta -ocimene	8.531	-	1.35	-
Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-cis	9.103	-	10.09	-
Linalool	9.739	-	1.37	-
Bicyclo [3.1.0] heptan-3-ol, 6,6 dimethyl-2-methylene	11.861	-	0.53	-
	11.283	-	2.8	-
Bicyclo [3.1.0] hexane, 4-methylene1(methyletyl)	12.382	-	1.25	-

AC4: Aframomum subsericeum, AC7: Aframomum alboviolaceum

Antiandrogenic effects of two Aframomum leaf essential oils in castrated male Wistar rats

In comparison to the SHAM-operated group, castration resulted in a significant regression of the prostate gland, seminal vesicle, Tyson gland, gland, and elevator muscles.

The relative wet weight of all the aforementioned organs increased significantly when castrated animals were given testosterone for ten days, with the exception of the prostate gland, where the rise was not statistically significant. Similar to casodex, 200 mg/kg of *A. subsericeum* leaf essential oils caused a substantial drop in the weight of the

gland, the seminal vesicle, and the prostate, as well as a nonsignificant decrease in the weight of the elevator muscles and Tyson gland as compared to the testosterone group. Nevertheless, the seminal vesicle's weight decreased significantly in response to the 100 mg/kg dose of *A. subsericeum*. Following the same trend as casodex

and *A. subsericeum*, *A. albobviolaceum* oil at a dose of 100 mg/kg significantly led to the regression of the prostate, the Tyson gland, and elevator muscles compared to the negative control (TESTO). On the other hand, the prostate weight was likewise dramatically decreased by the 200 mg/kg dose of *A. albobviolaceum* oil (Table 2).

Table 2: Effects of *A. subsericeum* and *A. albobviolaceum* leaf essential oils on the wet weight of androgen dependent organs.

	Prostate	Seminal vesicle	Gland	Tyson gland	Elevator muscles
SHAM	170.22± 11.19	1183.40±47.51	529.86±2.5	601.25±27.58	1002.306±26.90
CAST	111.26±1.30###	440.50±27.75###	273.86±14.86###	388.79±23.67###	506.78±11.309###
TESTO	117.80±1.79	619.96±45.79**	337.77±11.53*	463.76±32.02*	691.75±18.24**
CASODEX	82.45±7.58***	473.28±10.60*	257.21±8.89**	339.20±5.71	592.93±12.80
AC410	108.64±3.35	623.98±41.66	315.29±14.03	508.103±11.12	678.807±14.11*
AC4100	117.93±3.60	474.37±45.45*	279.18±27.11	405.98±24.16	584.35±13.13*
AC4 200	92.44±2.15***	409.67±16.04***	239.83±8.97***	399.44±19.95	640.046±21.04
AC710	102.97±4.53	511.58±15.77	301.67±19.04	434.93±15.70	691.24±4.36
AC7100	96.92±1.29*	607.01±14.23	301.215±22.77	365.68±8.82**	583.49±49.32*
AC7200	102.44±2.44*	516.17±13.57	295.04±3.79646	410.26±5.99	624.55±3.29

Data are expressed as mean ± SEM, n=5. SHAM: Sham operated animals which received 0.1% tween, CAST: castrated animals with 0.1% tween and corn oil. TESTO: = castrated animals co treated with testosterone enanthate (0.4 mg/kg) and 0.1% tween; CASODEX: positive control castrated animals co-treated with testosterone enanthate and casodex 3 mg/kg; AC4 and AC7: castrated animals co-treated with testosterone enanthate (0.4 mg/kg) and *A. albobviolaceum* or *A. subsericeum* essentials at doses of 10, 100 and 200 mg/kg BW; ### p<0.01 significant difference compared to the normal control (SHAM) *p<0.05; ** p<0.01; *** p<0.001: significant difference compared to the negative control

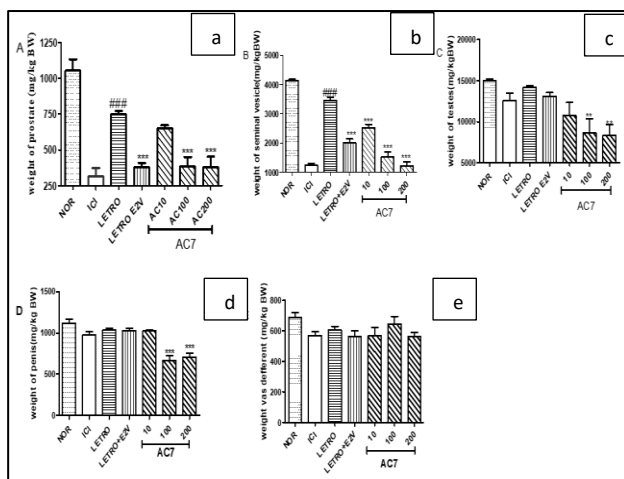


Figure 3: Effects of *Aframomum albobviolaceum* leaf essential oils on the wet weight of the prostate (A) seminal vesicle (B), testis (D), vas deferent (E) and penis (F).

Data are expressed as mean ± SEM (n=5). NOR =normal control animals treated with 0.1% tween; ICI = animals treated with fulvestrant (300 µg/150 gm); LETRO = negative control animals treated with letrozole (0.5 mg/kg); LETRO+E2V animals co-treated with letrozole (0.5 mg/kg) and estradiol valerate (400 µg/kg); AC7: animals co-treated with letrozole, estradiol valerate and *A. albobviolaceum* leaf essential oils at doses of 10, 100 and 200 mg/kg BW. ###p<0.01 significant difference compared to the normal control, **p<0.01; ***p<0.001: significant difference compared to the negative control LETRO.

Synergic estrogenic effects of *Aframomum* leaf essential oils on androgen dependent organs

The estrogenic effects of AC7 essential oils on androgen-dependent organs are illustrated in Figure 3A-F. Letrozole caused a non-significant drop in the wet weight of the testis, vas deferent, and penis, but a significant (p<0.001) decrease in the wet weight of the prostate and seminal vesicle when compared to the normal control. When compared to the letrozole animals, the prostate and seminal vesicle showed significant (p<0.001) decreases following the administration estradiol valerate. Compared to rats given only letrozole, the weight of the androgen-dependent organs decreased more when AC7 essential oils were supplied (Figure 3).

DISCUSSION

The GC/MS analysis presented in figures 1 and 2 and table II identified that the oils are mostly made of labdane terpenoids. The main compounds of *A. subsericeum* were γ-terpine (12.22%), cyclohexene, 1-methyl-4-(1-methylethylidene) (10.09%), terpinen-4-ol (10.01%), and those of *Aframomum albobviolaceum*: γ-Terpinene (20.07%), caryophyllene (11.58), myrtenyl acetate (7.22%), and (1R)2, 6, 6 trimethylbicyclo [3.1.1] hept-2-ene (6.97%). This composition is close to that of the *A. sulcatum* study in Cameroon, with β-caryophyllene (23.3%) as the major compound.¹⁶

Antiandrogenic substances are of great help in the treatment of many male disorders, including prostate tumors. This is because the prostate gland is sensitive to androgens, which influences its growth.¹ The results of this piece of work reveal that the castration of animals led to a decrease in the androgen-dependent organs compared to SHAM animals. These results are in line with the description of Hershberger Assay Guideline 890.1400 (OECD 2009).¹⁴ Other investigators also witnessed a significant drop in the weight of the prostate gland in castrated rats.¹⁷ In fact, the testis is the main source of androgens in the body, so castration lowers testosterone levels and leads to the regression of this organ. The injection of testosterone into castrated rats for 10 days led to an increase in the weight of these organs. Many studies carried out *in vivo* have shown that exogenous testosterone leads to an increase in the weight of androgen-sensitive organs.^{17,19} Casodex as well as *A. subsericeum* essential oils counteracted the effects of testosterone by leading to a decrease in the weight of these organs compared to the testosterone group. The decrease was more pronounced in the prostate gland. These results suggest that *A. subsericeum* essential oil has antiandrogenic properties. A study carried out *in vitro* on testosterone-stimulated SEC14L2 expression also reveals that essential oils from lavender show antiandrogenic properties.²⁰ However, Aframomum essential oils have been proven to have diterpenoids, which are responsible for the anti-androgenic effect observed. Interestingly, many studies have revealed the antiandrogenic properties of diterpenoids.²¹⁻²²

Exogenous oestrogen, through a negative feedback loop on the hypothalamus pituitary gland loop, is a potential strategy for achieving castrate levels of testosterone and therefore has been used to manage prostate cancer and avoid the physiological effects of oestrogen depletion. Only AC7 was used in this test because of its high yields, its abundance in almost every Cameroon locality, and because its androgenic properties were stronger at the dose of 100 mg/kg compared to those of AC4, which are stronger at the dose of 200 mg/kg. The results obtained from this investigation reveal that chemical castration of animals using the anti-aromatase enzyme letrozole and blocking of androgen receptors using ICI led to a significant decrease in the weight of androgen-dependent organs compared to normal animals. These results are in line with many scientific studies that show that letrozole administration to male rats reduces the weight of androgen-dependent organs.¹⁵ Fulvestrant, though an alpha-oestrogen receptor antagonist, has shown antitumor properties in prostate cancer through beta receptor regulation.²³ In fact, oestrogen is produced from androgens by aromatization. So, administration of letrozole lowers oestrogen levels and leads to the regression of these organs. The co-administration of letrozole and estradiol valerate led to a further decrease in the above organs compared to the letrozole animals. This atrophy of reproductive organs following estradiol administration is indicative of a competitive impairment of the AR receptor's function. Exposure of developing rats to estradiol has been seen to

modulate androgen receptors in the prostate. Also, oestrogens modulate AR in breast cancer.²⁴ Also, many studies have revealed that the administration of exogenous oestrogens in the presence of endogenous androgens leads to the atrophy of androgen-dependent organs, especially the testis. The administration of AC7 essential oils led to a more diminishing effect on the weight of androgen organs compared to the letrozole-treated animals. This shows that the essential oils possess a synergic estrogenic effect in male Wistar rats. We suggest that the presence of monoterpenes like diterpenoids are responsible for these estrogenic properties. Many results *in vivo* and *in vitro* reveal the estrogenic effects of essential oils.^{26,27} The regression of these organs with *A. alboviolaceum* essential oils could also suggest estrogenic effects of the essential oils through their selective binding to ER β . Many studies *in vivo* and *in vitro* prove that essentials have a high affinity for ER β .²⁶ ER β mediates regressive activities in androgen-dependent glands, especially the prostate.

CONCLUSION

The present study identified for the first time the chemical composition, antiandrogenic and estrogen synergic properties *A. subsericeum* and *A. alboviolaceum* leaf essential oils. The GC/MS results reveal that essential oils were mostly made of monoterpenes and diterpenes with γ -terpine and terpinene being the most abundant in *A. subsericeum* and *A. alboviolaceum* respectively. *In vivo* results reveal that both *A. subsericeum* and *A. alboviolaceum* antagonised the effects of testosterone on androgen dependent organs. While *A. alboviolaceum* show synergic action with oestrogens on the same organs. This antiandrogenic and oestrogenic synergic effect of Aframomum leaf essential suggests that the oils could provide a safety natural drug for prostate cancer treatment and management androgen of deprivation side effect.

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Conflict of interest: None declared

Ethical approval: All research protocols were approved by the Joint Institutional Review Board for Animal and Human Bioethics (Ref No: BTC-JIRB2023-078)

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