Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/13463
DOI URL: http://dx.doi.org/10.21474/IJAR01/13463

RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND APHRODISIAC EFFECT OF ETHANOLIC EXTRACT OF MASSULARIA ACUMINATA(G. DON) BULLOCK EX HOYL. (RUBIACEAE)IN MALE WISTAR RATS

Moussa Gbogbo[1], Alain Hugues Olivier N Guessan[2], Mama Koné[3], Stanislas Assom Ahoulou[1], Félicité Tano-bla Aboli[1] and Paul Angoué Yapo[3]

1. Department of Biochemistry and Microbiology, Jean Lorougnon Guédé University.
2. Laboratory of Bio-Organic Chemistry and Natural Substances (LCBOSN), Nangui Abrogoua University.
3. Laboratory of Physiology, Pharmacology and Pharmacopoeia, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire.

Manuscript Info

Manuscript History
Received: 28 July 2021
Final Accepted: 31 August 2021
Published: September 2021

Key words:-
Massulariaacuminata, Phytochemical Screening, Aphrodisiac, Rat

Abstract

Objective: The objective of the present study is to determine, through phytochemical screening, the major chemical compounds and to evaluate the aphrodisiac effect of the ethanolic extract of M.acuminata stems in adult male rats.

Methods : To achieve our objectives, a phytochemical study was conducted to determine the major chemical compounds present in the ethanolic extract of M. acuminatastems. The phytochemical screening was carried out following standard analytical procedures using thin layer chromatography.The aphrodisiac effect was evaluated in sexually naïve male rats. In sexually naïve male rats, a single administration of the ethanolic extract was performed at doses of 10, 20 and 40 mg/kg versus 0.5 mL/100g distilled water and 715 µg/kg Sildenafil Pfizer 50 mg in the negative and positive control rats, respectively. Female rats were induced to oestrus by sequential administration of estradiol benzoate (Sigma - Aldrich) (25 µg/rat) to make them receptive to males.

Results :The results of the phytochemical screening revealed that the ethanolic extract of M. acuminata stems contains coumarins, flavonoids, tannins, triterpenes of triterpenesaponins. The biological study showed that at doses of 20 and 40 mg/kg, ethanolic extract of M. acuminatastems resulted in a significant ($p < 0.05$) and highly significant ($p < 0.001$) increase in the number of sexual mounts, the number of erections, the number of ejaculations and a significant ($p < 0.01$) decrease in the latency time between sexual mounts in male rats.

Conclusions: The sexual stimulating effects of the ethanolic extract of M. acuminata observed in this study could be attributed to the presence of the identified chemical compounds, hence the interest in using this plant in traditional aphrodisiac medicine.

Corresponding Author:- Moussa Gbogbo
Address**:-** Department of Biochemistry and Microbiology, Jean LorougnonGuédé University.

Introduction:-

Sexual dysfunction (SD) is characterised by a disturbance in the processes that make up the sexual response or by pain associated with sexual intercourse (1). In men, SD mainly includes decreased sexual desire, ejaculatory disorders and especially erectile dysfunction (ED).

Hypoactive sexual desire in men can be due to somatic factors not specifically related to sexuality, to specifically sexual factors and to relational factors. One of the factors often cited as affecting sexual desire is age. Epidemiological studies have shown that sexual desire reduction has a high prevalence in the general population, thus representing an important cause of clinical sexology consultations. According to a study carried out in 29 different countries, 12.5-28% of men suffer from occasional, periodic or frequent sexual desire reduction and 1.3-3.9% of men suffer from frequently. In contrast, ejaculatory and orgasmic disorders apart from premature ejaculation are uncommon (2).

ED remains the most common sexual disorder. It was defined at the Second International Consultation on SD in 2004 as: "the persistent or recurrent inability of a man to achieve or maintain an erection sufficient for satisfactory sexual activity" (3).Although still taboo in some parts of the world, it affects more than 150 million men (4), and is estimated to be more than 300 million by 2025 (5), with a large increase in developing countries, particularly in Africa, Asia and South America.

Of psychological, neurological, hormonal or vascular origin, ED affects men of all ages, but mainly from 40 to 70 years of age.

The treatment of SD, apart from psychosexual follow-up, is nowadays essentially based on hormonal replacement drug treatments or the use of oral phosphodiesterase 5 inhibitors (IPDE-5) such as Sildenafil (Viagra).

In addition to these modern treatments, a large part of the population uses medicinal plants from the local pharmacopoeia through numerous recipes known in our societies. Among these plants is *Massulariaacuminata* (G. Don) Bullock ex Hoyl, a Rubiaceae. This plant species extends from Guinea Conakry to Zaire (6).

In Côte d'Ivoire, the ethanolic macerate of the stem bark of *M. acuminata* is used as a sexual stimulant and sold in cabarets under the name "4h du matin".

Despite testimonies on the supposed aphrodisiac effects, no study has yet confirmed this biological activity on male sexual function. It is in this perspective that the present study aimed to determine through phytochemical screening the major chemical compounds and to evaluate the aphrodisiac effect of the ethanolic extract of *M. acuminata* stems in adult male rats.

Materials and Methods:-

Plant material

Stems of Massulariaacuminata were collected in Bonoua, in the department of Grand-Bassam in the Sud-Comoé region (Côte d'Ivoire), 62 km from Abidjan, during November 2020. The plant was identified at the National Floristic Centre of Félix HouphouëtBoigny University, where a sample is registered under the number UCJ15291.

Animals

Male and female rats of the species *Rattusnorgevicus* of the Wistar strain, aged twelve (12) weeks and weighing on average between 150 g, were used for the experiment. They were raised in the animal house of the Laboratory of Physiology, Pharmacology and Pharmacopoeia of the Training and Research Unit of the NanguiAbrogoua University. They were fed with a standard diet and had free access to water with a nocturnal-diurnal (12/12) lighting rhythm. This study was approved by the ethics committee of the NanguiAbrogoua University.

Preparation of the ethanolic extract

Two hundred grams (200 g) of the powder obtained was macerated in 2.5 mL of 96% (v/v) ethanol for 24 h under continuous stirring. The macerate obtained was filtered and then concentrated under reduced pressure at 40°C using a rotary evaporator to obtain an ethanolic extract of *M.acuminata* stems.

Preparation of selective extracts

The dry extract obtained after concentration of the macerate under reduced pressure was dissolved in 60 mL of water to obtain an aqueous extract, which was successively exhausted with chloroform (3×20 mL) and ethyl acetate (3×20 mL). Thus, two selective extracts were obtained: Ec for the chloroform extract and Ea for the ethyl acetate extract.

Phytochemical screening

Saponindetection test

The test for saponins was performed according to the method described by Dohouet al.(7). One (01) g of dry *M.acuminata* stem powder was boiled for 30 min in 100 mL of distilled water. After cooling and filtration, the volume of the decoctate was readjusted to 100 mL with distilled water. In 10 test tubes of the same height and internal diameter, 1, 2, 3, ..., 10 mL of decoctate are successively introduced. The final volume is readjusted to 10 mL with distilled water. Each tube is shaken vigorously for 15 s. After 15 min of standing upright, the height of the persistent foam is measured. If the height of the foam is less than 1 cm in all tubes, the index is less than 100. If the height of the foam is equal to 1 cm in one of the tubes, the foam index (Im) is calculated according to the following formula :$I_m = \frac{1 \times V_x}{m_x}$

Vx: Volume of decoctate in the xthtube ;mx: Mass of plant material in the xth tube.

If the height of the moss is greater than 1 cm in all tubes, the index is greater than 1000. The presence of saponin in the stem of *M.acuminata* is confirmed, if Im is greater than or equal to 100.

Thin layer chromatography (TLC)of selective extracts

Phytochemical screening by TLC was performed on chromatographic plates, with an aluminium support (60 F254, 20×20, Fluka-Silica gel/DC), according to the analytical procedures described in several research works (8). The eluent used for the migration of the secondary metabolites contained in the selective extracts was a mixture of solvents consisting of Chloroform ($CHCl_3$)/Ethyl acetate (AcOEt)/Formic acid (HCOOH) in the proportions 6 /7 /2 (v/v/v).

Effects of ethanolic extract of *M. acuminata* on sexual activity of male rats

This study was conducted according to the method described by Carro-Juarez (9) with slight modifications. For this purpose, five (5) batches of five (5) sexually inactive male rats were treated orally one hour before the experiment as follows:

1. the negative control received 0.5 ml of distilled water per 100 g body weight;
2. the positive control received the reference molecule Sildenafil Pfizer 50 mg at a dose of 715 µg/kg;
3. test groups 3,4 and 5 received ethanolic extract at 10, 20 and 40 mg/kg respectively.

Female rats were induced to oestrus by sequential administration of estradiol benzoate (Sigma - Aldrich) (25 µg/rat) and subcutaneously, 48 h before the experiment. Estradiol benzoate was dissolved in peanut oil (Sigma-Aldrich) and injected subcutaneously in a volume of 0.1 ml/rat. The receptivity of female animals was confirmed prior to the test by exposing them to males, other than control and test animals. The most responsive females to the stimuli were then selected for the study (10).

The following sexual parameters were observed on each rat for one hour:

1. the number of sexual mounts, which represents the number of times the male mounts the female;
2. the number of erections, considered as the number of times the male licks his penis out of the prepuce;
3. the number of ejaculations, characterised by an ascent, a grasp, an intromission of the male's copulatory organ and the presence of a few drops of semen on the vaginal walls;
4. the latency time, which measures the time interval between two consecutive sexual climaxes (expressed in seconds).

Statistical analysis

Data were expressed as mean ± standard error on the mean of five replicates. Means were analysed using a one-factor ANOVA followed by a Dunnet's post hoc test (multiple comparisons test) to determine significant differences between the mean of the control and the means of the other test groups.

All statistical analyses were performed using XLStat Pro 2015.1.02 software (Addinosoft, Bordeau, France) while graphs were plotted using the Excel program. Significance was defined at the threshold of $p < 0.05$.

Results:-

Determination of the moss index

The height of the moss in the ten tubes being greater than 1 cm means that the moss index of the plant material is greater than 1000. This indicates an abundant presence of saponins in the stems of M.acuminata.

Identification of secondary metabolites of selective extracts

The identification of phenolic compounds by TLC of the Ea and Ec extracts showed the presence of coumarins, flavonoids, terpenes in all the extracts analysed except for the Ea extract in which the additional presence of tannins was identified (Figure 1).

This presence or copresence of flavonoids was observed under Ultraviolet light (UV) 365 nm, without developer, and then confirmed by aluminium chloride ($AlCl_3$), a specific reagent for flavonoids. Indeed, $AlCl_3$ reveals them under UV 365 nm, in colours ranging from blue to brown or yellow-green. These fluorescences are marked by retention factor (Rf) of 0.03 (yellow-green), 0.07 (yellow-green); 0.08 (yellow-green); 0.61 (blue); 0.67 (blue); 0.73 (blue); 0.90 (yellow) for Ea and 0.03 (blue); 0.07 (blue); 0.08 (blue); 0.61 (yellow); 0.67 (blue); 0.73 (yellow); 0.9 (yellow) for Ec (Figure 1A). Coumarins are revealed by potassium hydroxide (KOH) (5%, m/v) in blue and green spots (8). These are marked by Rf of 0.00 (green); 0.03 (green); 0.08 (green); 0.13 for both extracts and 0.73 (green for Ea and fluorescent green for Ec) (Figure 1B). For tannins, they appear as grey spots, the case of the Ea fraction (Rf = 0.00; 0.03; 0.08). As for terpenes, their presence is revealed by Godin's reagent in purple spots, materialized on figure 1E by Rf of 0.03; 0.07; 0.08; 0.13; 0.17, 0.27 for Ea and a trail going from 0.03 to 0.67 and Rf of 0.67; 0.73 for EC.

From the results of the TLCof the selective extracts of the stems of *M.acuminata* and the moss index test, it appears that the stems of this plant contain coumarins, flavonoids, tannins, triterpenes and triterpenesaponins.

Evaluation of the properties of ethanolic extract of *M.* acuminata stems on sexual parameters of male rats

Effect of the extract on the number of sexual mounts

At 20 mg/Kg, ethanolic extract of *M. acuminata*stems induced a non-significant increase in sexual mounts compared to the negative control (29.4 ± 7.02 vs 7.2±1.9; $p>0.05$) (Figure 2). In contrast, at a dose of 40 mg/Kg body weight, the extract induced a highly significant increase in sexual mounts compared to the negative control (79±26.7 vs. 7.2±1.9; $p<0.001$). At the same dose, sexual mounts were slightly higher than those observed in positive control rats treated with the reference molecule (79±26.7 vs. 69.4±20.58; $p < 0.05$) (Figure 2).

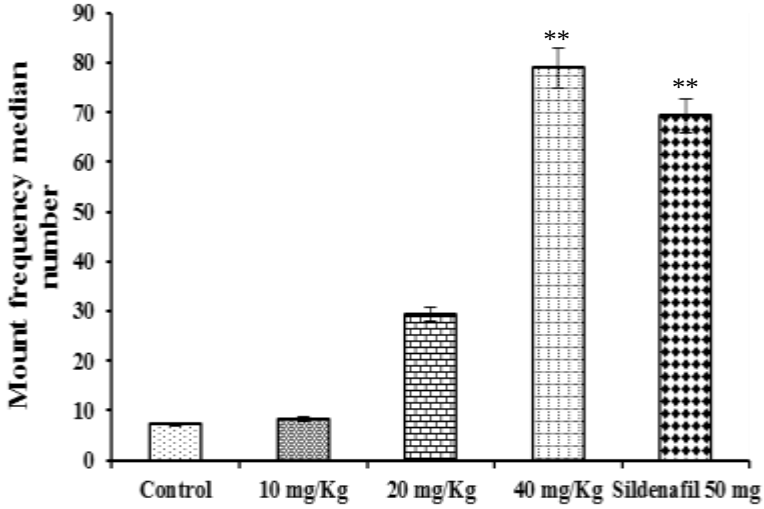

Figure 2:- Effect of ethanolic extract of M. acuminata stems on the number of sexual mounts in treated male rats; n = 5; *: significant increase ($p < 0.05$); **: highly significant increase ($p < 0.001$) compared to the negative control.

Effect of the extract on the number of erections

Administration of the ethanolic extract at dose of 10 mg/Kg body weight did not alter erectile behaviour in rats compared to the negative control (5.8±3.9 vs. 7.8±3.5; p> 0.05) (Figure 3). However, at the respective doses of 20 and 40 mg/Kg, the extract caused significant and highly significant increases in the number of erections in treated rats compared to the negative control (25.6±6.8 vs. 7.8±3.5; p<0.05 and 60.6±12.6 vs. 7.8±3.5; p<0.001). Again, the number of erections was significant with no significant difference in the group treated with 40 mg/Kg of the ethanolic extract than the effect of the reference molecule (60.6±12.6 vs 53.2±8.3, p> 0.05) (Figure 3).

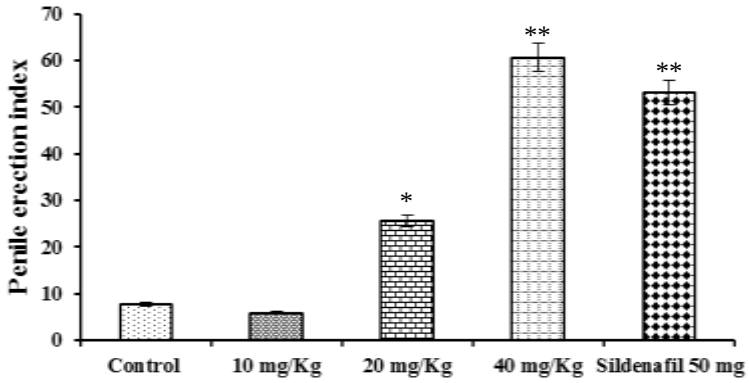

Figure 3:- Effect of ethanolic extract of *M. acuminata* stems on the number of erections in treated male rats; n = 5; *: significant increase (p < 0.05); **: highly significant increase (p < 0.001) compared to the negative control.

Effect of the extract on the number of ejaculations

A significant increase in the number of ejaculations was observed in rats treated with the ethanolic extract of *M. acuminata*stems at doses of 20 and 40 mg/Kg body weight compared to the negative control group (2.4 ± 0.5 vs. 0.6 ±0.5; p<0.05 and 3.2 ±1.3 vs. 0.6 ±0.5; p<0.05) (Figure 4). The positive control group also showed a significant increase in the number of erections in rats compared to the negative control group (3.6 ±1.5 vs. 0.6 ±0.5; p<0.05) (Figure 4).

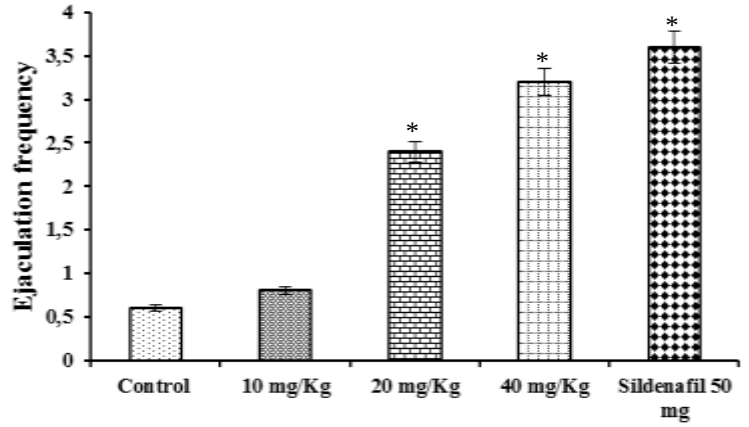

Figure 4:- Effect of ethanolic extract of *M. acuminata* stems on the number of ejaculations in treated male rats; n = 5; *: significant increase (p < 0.05) compared to the negative control.

Effect of the extract on the latency time between two consecutive sexual mounts

A highly significant (p<0.001) decrease in the latency time between two consecutive sexual mounts was observed in the groups of rats treated with the ethanolic extract at doses of 20 and 40 mg/kg body weight (respectively 118.8±10.2 vs. 442±33.0; and 42.6 ± 6.5 vs. 442±33.0) and the reference molecule (48.8 ±11.3 vs. 442±33.0) (Figure 5). For the dose of 10 mg/Kg body weight, the ethanolic extract had a similar effect to that caused by the administration of distilled water (432.2 ± 10.2 vs 442±33.0; p > 0.05) (Figure 5).

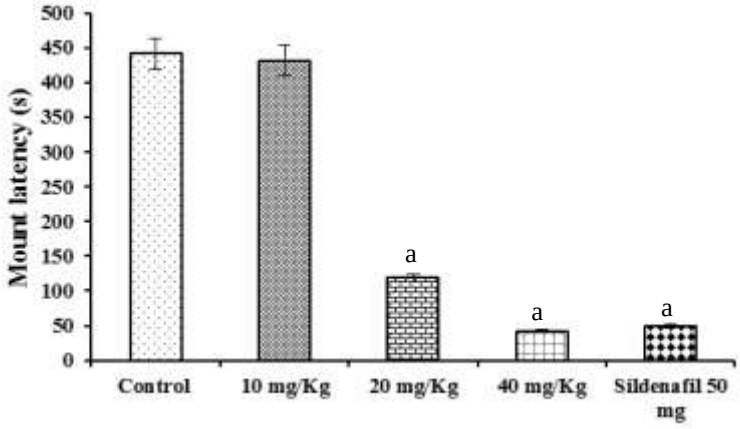

Figure 5:- Effect of ethanolic extract of *M. acuminata* stems on latency between sexual mating in treated male rats; n = 5; a: highly significant decrease ($p < 0.001$) compared to the negative control.

Discussion:-

The phytochemical study revealed in the ethanolic extract of the stems of *Massulariaacuminata*(Rubiaceae) the presence of coumarins, flavonoids, triterpenesaponins, tannins and triterpenes.There are nearly a thousand coumarins described in more than 800 plants species, including the Rubiaceae (11). According to some authors, coumarins have numerous pharmacological properties, including antioxidant activity and vasodilatation (12). Contrary to our result, these authors (13) did not observe the presence of coumarins during their phytochemical study on the hydroalcoholic extract of the trunk bark of *M.acuminata*. This result could be explained by the different nature of the solvent but also by the plant organ used.

Flavonoids, triterpenesaponins and tannins were also observed in the aqueous and hydroalcoholic extracts of *M. acuminata* trunk bark in the work of Maloueki (13). Flavonoids have numerous activities: antioxidant, enzyme inhibiting, vasodilating (14). The triterpenesaponins contribute to sexual stimulation activity by increasing the level of androgens (15).

This study also showed good sexual activity in male rats treated with the ethanolic extract of the stems of *M. acuminata* (Rubiaceae). Indeed, a significant increase in the number of sexual mounts was observed in male rats treated with the extract at dose of 40 mg/Kg compared to the negative control. At the same dose, the number of sexual mounts was also higher compared to the positive control treated with Sildenafil 50 mg. These results suggest that the ethanolic extract would increase sexual appetite and exert a tonic effect one hour after its intake. Our results are similar to those obtained by (16). These authors showed that administration of the aqueous extract of the roots of *M. acuminata*(Rubiaceae) at dose of 50 mg/Kg of body weight significantly increased the sexual drive in test rats compared to control rats. The increase in libido in rats is thought to be mediated by the presence of flavonoids in the extract, as this compound directly affects male function (17) by increasing vasorelaxation of cavernous smooth muscle cells by activating the cyclic nitric oxide guanosine monophosphate (NO-cGMP) pathway (18), or by interacting with central pathways that participate in libido or spontaneous sexual arousal.

This study also showed a significant and highly significant ($p<0.05$ and $p<0.001$) increase in the number of erections as well as in the frequency of ejaculation in rats with the ethanolic extract compared to negative control rats. These results are similar to those of (16) who observed a significant increase in the number of erections and frequency of ejaculations in rats treated with the aqueous extract of *M.acuminata* at doses of 50, 100 and 200 mg/Kg. According to some authors, erection occurs as a result of nervous system-mediated relaxation of penile smooth muscle (19). The presence of flavonoids, coumarins and triterpenesaponins may account for the increased frequency of erections in this study. These chemical compounds have been shown to act indirectly and favourably on blood flow to the penile erectile and ejaculatory structures.

The highly significant ($p<0.001$) decrease in the latency time between sexual mounts suggests a copulatory performance of rats treated with ethanolic extract at doses of 20 and 40 mg/Kg body weight compared to control rats. This performance was reflected in a very short post-ejaculatory recovery time. These results are comparable to those obtained by (20) who observed a significant decrease ($p < 0.05$) in the latency time between sexual mating in rats treated with the aqueous extract of the roots of *M.acuminata* at doses of 50 and 100 mg/Kg compared to the

control group. The ethanolic extract of the stems of *M.acuminata*would thus improve physical performance during sexual intercourse by reducing nervous fatigue caused by successive mating.

Conclusions:-

The sexual stimulating effects of the ethanolic extract of *M. acuminata* observed in this study could be attributed to the presence of the identified chemical compounds, hence the interest in using this plant in traditional aphrodisiac medicine.

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