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Comparative phytochemical screening of *Ereromastax speciosa* and *Ereromastax polysperma*

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ABSTRACT

In this study, the phytochemical constituents of Ereromastax speciosa and ereromatax polysperma leaves was investigated. These leaves are commonly used amongst traditional birth attendants in the rural regions of calabar cross river state and akwa ibom state, Nigeria. In this study, experienced traditional birth attendants from the local regions in cross river and akwaibom were orally interviewed about the use of these herbs in enhancing fertility. Claims have been made by these traditional birth attendants on the efficacy of these plants for the treatment of internal heat, arresting of post partum bleeding, other reproductive complications like retention of placenta, enhancing of fertility in infertile females. Quantitative phytochemical analysis of these leaves were determined using the methods described by Harborne [1], including the method of Swain[2] for the determination of tannins , Spectrophotometric method of Brunner [3] for the determination of saponnins, Folin–Ciocalteau reagent method[4], for the determination of polyphenols, has revealed the presence of bioactive components in varying quantities. Results on phytochemical analysis on Ereromastax polysperma and Ereromastax speciosa respectively showed the presence of Tannins(15.00±0.00 and 45.00±0.00), phenols(55.00±0.00 and 10.50±1.50), flavonoids(977.50±27.50 and 1247.50±2.50), alkaloid s(652.50±2.50 and 202±2.50), saponins (975.00±0.00 and 75.00±0.00), terpenes(1075.00±25.00 and 80.00±5.00) . The findings from this study show the therapeutic values of these leaves and its use in the management of female infertility.

Keywords: fertility, phytochemicals, ereromastax speciosa, and *Ereromastax polysperma*.

INTRODUCTION

The medicinal plants ereromastax speciosa, and *Ereromastax polysperma*, locally known as ikpo ikong and edem iduduot respectively amongst the efiks and ibbibios in akwa ibom and cross river state belong to the acanthaceae family. They are tropical stout erect multibranched herbs[5], these herbs are mostly found in the tropical regions of Nigeria and Cameroon. They are grown in the farmyards of most rural dwellers for medicinal and ornamental purposes.

Ereromastax speciosa along with its fellow genre plant *Ereromastax polysperma* in combination with other herbs have been in use by traditionalist in the local regions of calabar and akwa ibom to treat women with fertility problems. Traditionalists by nature do not have a documented record of their herbal remedies, rather the knowledge on the use of these herbs are passed orally from previous generations [6].

Reports have shown that extracts from the herbs of E. speciosa has been employed in the treatment of dysentery, anaemia, diarrhoea,[7] irregular, and urinary tract infection [8].

E. polysperma plant, is commonly called the blood tonic plant, in the treatment of diabetes, anaemia, and internal heat the plant is mixed with eggs [9] and in the treatment of penfigures in children[10].

Maternal related conditions contribute to one of the leading causes of death followed by malaria and HIV/AIDS in developing countries [11] Women are increasingly using herbal remedies to combat fertility related conditions.[12]. In Nigeria, a survey conducted on 1200 pregnant women demonstrated that 12% used native herbs [13]. Infertility can be the source of a major life crises between couples [14] and this condition can lead to separation. Therefore there is the need to source for new drug potentials in medicinal plants that have been in use traditionally and to investigate their possible bioactive principles which can be used in the management of fertility issues in women. On this note, the phytochemical analysis on the medicinal plants this study, will contribute to knowledge in this area.

MATERIALS AND METHODS

Sample collection and preparation *Ereromastax speciosa* and *Ereromastax polysperma* leaves were identified and authenticated in the hebarium unit of the department of botany faculty of sciences in the university of calabar, and the forestry department cross river state. Thereafter, the fresh leaves were harvested from a traditional farm at akai effa calabar municipality. The fresh leaves were thoroughly washed and allowed to dry under shade. The dried leaves were blended into fine powder using a Q-link electrical blender model QBL-18L40. Two hundred and forty one (241.0g) blended E. speciosa and one hundred and eighty point ten (180.10g) blended E. polysperma were soaked in 2500mls of 98% ethanol as extracting solvent and allowed to stand for 72 hours at room temperature. The mixture was filtered using whatman No l filter paper to obtain a homogenous filtrate. The extract was concentrated in vacuo at a low temperature of (37-40˚c) using a rotary evaporator (Model RE52A, China). The concentrates yielded 28.3g and 16.4g respectively. The concentrated extracts were used for phytochemical analysis.

Phytochemical analysis

Quanitative phytochemical compositions of the leaves were determined using the methods described by Harbone [1], including the method of Swain[2] for the determination of tannins , Spectrophotometric method of Brunner [3] for the determination of saponnins, Folin–Ciocalteau reagent method [4], for the determination of polyphenols

Quantitative determination of alkaloids

This was done by the alkaline precipitation gravimetric method described by Harborne, [1]. A measured weight of the sample was dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4h at 28oC. It was later filtered via what man No 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of conc.aqueous NH4OH until the alkaloid was precipitated. The alkaloid precipitated was received in a weighed filter paper, washed with 1% ammonia solution dried in the oven at 800C. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

Quantitative Determination of Flavonoids

This was determined according to the method of Harborne [1]. 5gram of the sample was boiled in 50ml of 2M HCl solution for 30min under reflux. It was allowed to cool and then filtered through whatman No 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

Quantitative determination Tannins:

The method of Swain [2] was used for the determination of tannin content. 0.2 g of finely ground sample was measured into a 50 ml beaker. 20 ml of 50% methanol was added and covered with parafin and placed in a water bath at 77-80oC for 1 h and stirred with a glass rod to prevent lumping. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper into a 100 ml volumetric flask using 50% methanol to rinse. This was made up to mark with distilled water and thoroughly mixed. 1 ml of sample extract was pipette into 50 ml volumetric flask, 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na2C03 were added and mixed properly. The mixture was made up to mark with distilled water, mixed well and allowed to stand for 20 min when a bluish-green colouration developed. Standard Tannic Acid solutions of range 0-10 ppm were treated similarly as 1 ml of sample above. The absorbances of the Tannic Acid Standard solutions as well as samples were read after colour development on a Spectronic 21D Spectrophotometer at a wavelength of 760 nm. Percentage tannin was calculated using the formula:

Tannin (%) = Absorbance of sample x Average gradient x Dilution factor
Weight of sample x 10,000

Quantitative determination Saponin: The Spectrophotometric method of Brunner [3] was used for saponin analysis. 1 g of finely ground sample was weighed into a 250 ml beaker and 100 ml Isobetyl alcohol was added. The mixture was shaken on a UDY shaker for 5 h to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman No. 1 filter paper into a 100 ml beaker and 20 ml of 40% saturated solution of Magnesium carbonate added. The mixture obtained with saturated MgC03 was again filtered through a Whatman No 1 filter paper to obtain a clear colourless solution. 1 ml of the colourless solution was pipetted into 50 ml volumetric flask and 2 ml of 5% FeCl3 solution was added and made up to mark with distilled water. It was allowed to stand for 30 min for blood red colour to develop. 0-10 ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly with 2 ml of 5% FeCl solution as done for 1 ml sample 3 above. The absorbances of the sample as well as standard saponin solutions were read after colour development on a Spectronic 2lD Spectrophotometer at a wavelength of 380 nm.

Percentage saponin was calculated using the formula:

Saponin (%) = Absorbance of sample x Average gradient x Dilution factor
Weight of sample x 10,000

Quantitative Determination of Terpenes
1gram of each of the samples was added to 10 ml of petroleum ether and allowed to extract for 15mins. The solution was filtered and read at an absorbance of 420nm.

3.2.2.l0 Quantitative determination of Total polyphenols
Total polyphenols were determined according to the Folin–Ciocalteau reagent method [4]. Two-hundred microlitres of extracted sample, in triplicate, were added to 1 ml of 0.2 N Folin–Ciocalteau reagent and 0.8 ml of 7.5% sodium carbonate solution, mixed well and allowed to stand for 30 min at room temperature. Absorption at 765 nm was read using a Shimadzu 300 UV–Vis spectrophotometer. Quantification was based on the standard curve generated with 100– 400 mg/l of gallic acid.

RESULTS

Quantitative estimates in percent % in the leaves of *Ereromastax polysperma* and ereromastax speciosa.

	Tannin	Phenol	Flavonoids	Alkaloids	Saponins	Terpenes
Eremomastax Polysperma						
	15.00	55.00	977.50	652.50	975.00	1075.00
	±0.00	±0.00	±27.50	±2.50	±0.00	±25.00
Eremomastax Speciosa						
	45.00	10.50	1247.50	202.50	75.00	80.00
	±0.00*	±1.50*	±2.50*	±2.50*	±0.00*	±5.00*

Values are expressed in triplicates as ±SEM @ P<0.05

DISCUSSION

The phytochemical screening of the leaves of ereromastax plolysperma and *Ereromastax speciosa* revealed the presence of tannins, phenols, flavonoids, alkaloids, saponins, terpenes in varying quantities. The theurapeutic values of medicinal plants are attributed to their phytochemical constituents. The highest constituent of all the phytochemicals in the specie of *Ereromastax polysperma* was in the terpenes content(1075.00±25.00). Terpenes play an important role in cellular membrane fluidity, as a result of the triterpenes which serve as a precursor molecule for the cholesterol. Cholesterol is a precursor for steroid hormones like progesterone, estradiol, and testosterone. Estradiol and progesterone play a pivotal role in the functions of the female reproductive cycle, like ovulation, implantation and maintainance of pregnancy. The fallopian tube mainly functions as a passage for the transport of the released egg from the ovary to the uterus, without which there will be no implantation, this function is maintained by the healthy fallopian tubes. This therefore suggests that the plant extracts investigated in this study can serve as a potential drug source for the management of female fertility issues. Terpenes, a phytochemical, is often used as a potent drug component against life threatening diseases like malaria [15] heart disease[16]and cancer[17].

Ereromastax speciosa has its highest phytochemical constituent in the flavonoids(1247.50±2.50). Flavonoids are plant secondary metabolites that are widespread throughout the plant kingdom. Based on their backbone structure, flavonoids are divided into chalcone, flavonols and anthocyanins. Flavonoids have antioxidant anti microbial properties and as antioxidants they protect against oxidative damage. In flowers, flavonoids play a role in sexual reproduction and fertility, this is because inhibition of flavonoid production in plants through antisense suppression of the gene encoding chalcone synthase(CHS) resulted in the inhibition of flower pigmentation and male sterility[18].

The high quantity of terpenes and flavonoids in the phytochemical constituents of *Ereromastax polysperma* and speciosa plants suggests its posible use in relation to the vitex plant which is used in the treatment of fertility conditions in women. The vitex plant contains monoterpenes and the flavonoids in sufficient quantities, its beneficial effects can also be attributed to its phytochemical constituent[19]. The vitex plant is used to maintain a balance in the female reproductive hormonal system.

Other phytochemical constituents found in the *Ereromastax polysperma* and *Ereromastax speciosa* plant species includes the alkaloids. Alkaloids fall into one of the common groups of phytochemicals they are known to have a lot of physiological activities. Morphine was the first identified alkaloid from the opium poppy plant, it is used to treat acute and chronic severe pains, as well as pains resulting from myocardial infarction, and labour [20]. Capasaicin is a fiery alkaloid is usually associated with pepper plants, it posseses the ability to heat up the tongue and skin if touched. Studies have shown that capasaicin encourages the release of endorphins. It has also been used in skin ointments because it aids receptor cells in sensing heat and relieving minor pains and those caused by damage to the cells of the peripheral nervous system [21].

Polyphenols from medicinal plant extracts, include the isoflavones, flavones, quercitin, glucosides, anthrocyannins, have been identified to posses antioxidant activities . In addition, anticancer activities in cultured cells of the breast, colon, lungs and prostate has been detected in anthocyanins and quercetins, this is probably due to the protective functions of antioxidants[22] Quercetins promote cardiovascular health, inhibits inflammatory mediators and the release of histamines

Tannins found in some herbs give herbs their astringent characteristic taste, these herbs are used to treat intestinal disorders such as diarrhoea and dysentery. Tannins have been reported to react with proteins by forming irreversible complexes which is useful in the treatment of inflamed or ulcerated tissues, tannins also have anticancer activities [23]. Saponnins belonging to another class of phytochemicals have been reported to have antifungal and anti inflammatory effects [24]. Their amphiphylic property makes them useful surfactants invivo. They enhance the penetration of proteins through cell membranes [25]. Saponnins serve as useful aids as pharmacological and immunological agents in enhancing the recipients' immune's response to a supplied antigen [26]. Saponins are useful cholesterol lowering agents, they work in the digestive system by binding with bile and dietary cholesterol, this prevents cholesterol reabsorption thereby increasing its excretion [27]. Digitalis is a type of saponin that is used in the treatment of heart conditions. The expectorant property of saponin is used in the relief of cough, it increases bronchial secretion resulting in the dilution of sputum [28].

CONCLUSION

From this study, it can be said that the leaf extracts of ereromastax polysperma and *Ereromastax speciosa* contain some bioactive principles and are therefore therapeutically active as fertility enhancing plants. These pharmacological properties are attributed to their phytochemical constituents.

REFERENCES

[1]Harbone JB (**1973**). Phytochemical methods: A guide to Modern technique of plant analysis. Chapman and Hall, London, 279
[2]Swain, T(**1979**) Tanins and lignins In Rosenthal GA and Janzen DH (eds) . Herbivores their interaction with plant metabolites. Academic Press. New York.
[3]Brunner J. H (**1984**) *Anal. Chem*. 34 pp 1314 – 1326
[4]Singleton, V.L. and J.A. Rossi, **1965**. *Am. J. Enol. Vitic*., 16: 144-158
[5]Heine, M (**1962**). *FWTA*(ed.2), 2:397, Jstor, Plant Science
[6]Catherine, K, K, Jemimah A,O,Titus I ,Kanui (**2011**) practices of TBA's in Machakos District Kenya; *J. Ethnopharmacol*. Doi 10,101611/j.j ep2011.04405
[7]Oben, J. E., Assi, S. E., Agbor, G. A and Mosors, D. F. (**2006**). *Africa Journal of Traditional Medicine*. 3:95-100

[8]Adjanohoun, J. E., Abubakar, K., Dramane, M. E., Ebot, J. A., Ekpereband, E. G. (**1996**). Traditional medicine and pharmacopoeia, contribution to etnobotanical floristic studies in Cameroons. (NPMS,Benin, pp. 22)
[9]Pandey, B. P. (**2006**). Text book of botany angiosperms. S. Chad and company ltd. Ran Nagar, New Delhi.
[10]Bassey, M. E and Effiong, E. O(**2011**) *Journal of Natural Product Plant Resource* 1,(3):33-42```
[11]Who,**2006**, World Health Organisation **2006** *Journal of Ethnobiology and Ethnomedicine*. 3:13
[13] Gharoro, E. P and Igbafe, A.A.(**2000**) *Med Scientific Monitor*, 6,84-87
[14]Burns LH, Convington SH. (eds) Infertility counselling: a comprehensive handbook for clinicians. New York: Parthenon Publishing; **1999**
[15]Parshikov, I. A, A. I. Netrusov , J. B. Sutherland, Microbial transformation of antimalarial terpenoids, *Biotechnology Advances*, doi:10.1016/j.biotechadv.**2012**.03.010
[16]Liebgott, T, M. Miollan, Y. Berchadsky, K. Drieu, M. Culcasi and Sylvia Pietri, *Basic Research in Cardiology*, **2000**, 95, 5, 368 – 377
[17]Ebada, S.S, W.H. Lin and P. Proksch, *Marine Drugs* **2010**, 8, 313-346
[18]Van der Meer IM, Stam ME, Van Tunen AJ, Mol JN, Stuitje AR (**1992**) *Plant Cell* 4: 253–262
[19]Yarnell, Eric. "Home Remedies for Reproductive Health" 09 July **2007**. HowStuffWorks.com. <http://health.howstuffworks.com/wellness/natural-medicine/home-remedies/home-remedies-for-reproductive-health.htm> 10 January **2013**.
[20] AHFS-Drug information. (**2006**). *International Journals of Andrology*; 23:82-84
[21]PLoS Medicine(**2005**) Which Treatment For Post Herpertic Neuralgia.(PLos Med) 2 (7): e238. Doi10.1371/journal.pub med.0020238.
[22] Neto, C. C. (**2007**). *Journal of Nutrition*. 137(1):1865-1935
[23] Dharmananda, S. (**2003**). Gallnuts and the uses of tannins in Chinese Medicine In: proceedings for Traditional Medicine
[25] Sule, W. F., Okonko, I. O., Omo-Ogun, S., Nwaeze, J. C. Ojezele, M. O, Ojezele, O. J., Alli, Soyemi, E. T., Olanipekun, T. O. (**2011**). *Journal of Medicinal Plant Research*
[26] Liener, I . E. (**1980**) Toxic Constituents Of Plants Foodstuff. NewYork City Academic Press. p161, ISBN NUM-112-449860
[27] Skene, C.D & Phillips S.(**2006**). Saponin-adjuvanted particle vaccine for clinical use. Methods. 40(1):53-59.
[28] Kee, C. H. (**1998**). The Pharmacology Of Chinese Herbs . 2nd ed., ISBN 978-84923-16654