OBAFEMI AWOLOWO UNIVERSITY

# NEUROPHARMACOLOGICAL EFFECTS OF THE ESSENTIAL OIL AND AQUEOUS EXTRACT OF *CYMBOPOGON CITRATUS* (POACEAE) IN MICE


**BY**

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## CERTIFICATION

We hereby, certify that CHINWUBA Ekwutosi Patricia carried out this research work during the course of her study in the department of pharmacology of the faculty of pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

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**DEDICATION**

This work is dedicated to Jehovah El Roi and to everyone, everywhere who is struggling for any good cause and whose face is turned up-wards.

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| | |
|---|---|
| ACh | Acetylcholine |
| ACTH | Adrenocorticotropic hormone |
| AMPA | Alpha-amino-3-hydroxyl-5-methylisoxazolepropionic acid |
| BZDs | Benzodiazepines |
| CCK | Cholecystokinin |
| CNA | Central nucleus of amygdala |
| CNS | Central nervous system |
| GABA | Gamma amino butyric acid |
| GAD | Generalized anxiety disorder |
| HPA | Hypothalamic-pituitary-adrenal-axis |
| LA | Lateral nucleus of the amygdale |
| $LD_{50}$ | Lethal dose for 50% of the population |
| MAOIs | Monoamine Oxidase Inhibitors |
| MHPG | 4-Methoxy-3-Hydroxy-Phenyl-Glycol |
| NE | Norepinephrine |
| NIBs | Novelty-induce behaviours |
| NIG | Novelty-induced grooming |
| NIR | Novelty-induced rearing |

| | |
|---|---|
| NMDA | N-methyl-D-aspartate |
| NPY | Neuropeptide Y |
| OFL | Open-field locomotion |
| OCD | Obsessive-compulsive disorders |
| PANDAS | Paediatric Auto-immune Disorder Associated with Streptococcal |
| PD | Panic disorders |
| PGs | Prostaglndins |
| PTSD | Post traumatic stress disorder |
| SNRIs | Serotonin and Noradreanline re-uptake inhibitors |
| SSRIs | Selective Serotonin Inhibitors |
| WHO | World Health Organization |
| 5-HT | 5-hydroxytryptamine |

## ABSTRACT

This study determined the effects of the oil and aqueous extract of *Cymbopogon citratus* (Poaceae) Stapf on learning and memory, it further evaluated the anti-depressant and analgesic properties of the oil and aqueous extract in mice. This was with a view to providing information on the neuropharmacological effects of the oil and aqueous extract.

The essential oil of the plant was obtained by hydrodistillation using Clavenger apparatus while the aqueous extract was obtained using a reflux apparatus. Acute toxicity ($LD_{50}$) of both oil and extract were determined by Lorke's method for oral route only. The oil (50, 100, 150 and 200 mg/kg) and the extract (250, 500, 1000 and 2000 mg/kg) were individually evaluated for novelty-induced behaviours (locomotion, rearing and grooming) in open field, anxiolytic (hole board and elevated plus-maze), analgesic (hot plate model), anti-depressant (forced swimming test) and learning and memory (Y-maze) effects in mice. The data were analyzed using the GraphPad® primism 4.0 statistical software. The test doses were compared with control by one way analysis of variance (ANOVA) followed by post hoc analysis using Student Newman-Kuels multiple comparison tests.

The results obtained for acute toxicity studies indicate that the $LD_{50}$ of the essential oil (EO) and aqueous extract (AQ) of *C. citratus* were 948.68 and 5000 mg/kg, by the oral route respectively. The oil induced no significant ($p>0.05$) effect on novelty induced rearing (NIR) but the extract (1000-2000 mg/kg) caused significant ($F_{(5, 30)}= 7.95$; $p<0.05$) increase in locomotor activity when compared to control. The EO and AQ at all the doses caused significant increase in the number of head dips ($F_{(5, 30)}=12.63$; $p<0.05$), ($F_{(5, 30)}=15.88$; $p<0.05$) and increase in the time

spent in the open arms of the elevated plus-maze ($F_{(5, 30)}$=4.66; $p<0.05$), ($F_{(5, 30)}$= 9.96; $p<0.05$) respectively, indicating anxiolytic effects. The EO and the AQ at all the dose levels caused a significant ( $F_{(5, 30)}$= 23.25; $p<0.05$), ( $F_{(5, 30)}$= 76.82; $p<0.05$) reduction in the immobility time in the FST respectively, indicating anti-depressant effect. Finally at these dose levels, the EO and AQ significantly ($F_{(5, 30)}$= 14.55; $p<0.05$), ($F_{(5, 30)}$= 19.68; $p<0.05$) caused increase in the reaction time on the hot plate model respectively, indicating analgesic effects.

The study concluded that the essential oil and aqueous extract of *C. citratus* possessed significant anxiolytic, analgesic and anti-depressant properties.

# CHAPTER ONE

# INTRODUCTION AND LITERATURE REVIEW

## 1.1 Introduction

Ethnomedicine is the study of traditional medical practice which is concerned with the cultural interpretation of health, diseases and illness and also addresses the healthcare seeking process and healing practices (Krippner, 2003). The practice of ethnomedicine is a complex multi-disciplinary system constituting the use of plants, spirituality and the natural environ-ment and has been the source of healing for people for millennia (Lowe *et al*., 2000). The components of ethnomedicine have long been ignored by many medical practitioners for various reasons, including; the chemical composition, dosages and toxicity of the plants used in ethnomedicine are not clearly defined (Lowe *et al*., 2000).

The WHO estimates that up to 80% of the populations in sub- Saharan Africa make use of ethnomedicine (WHO, 2003). Indeed, in spite of widespread introduction of western-type medical services in sub-Saharan Africa; traditional medicine remains the most subscribed and accessible therapeutic system in the region. For instance, it is estimated that in Nigeria, ethnomedicine is actually the only healthcare resource accessible to a third of the population and scholars have attributed the popularity of ethnomedicine in Nigeria to its affordability, accessibility, cultural acceptability, and proven efficacy (Jegede, 1998; Udoh, 2000; Erinosho, 1989). The literature reports that Nigerians tend to view ethnomedicine as efficacious for most illnesses (Izugbara *et al*., 2005; Modo, 2005). A major attraction of traditional medicine in Nigeria may also be that it always has some kind of cure available for virtually any condition in

contrast to the allopathic practitioners who might prescribe medications not easily attainable (Outwater et al., 2001; Izugbara *et al*., 2005).

The plant genetic resources of Nigeria, are a veritable source of pharmaceuticals and therapeutics, though the plants are not adequately documented. Medicinal plants are generally scattered in various families of angiosperms, gymnosperms, pteridophytes, bryophytes and thallophytes.

It is interesting to note that the ethnomedicinal use of plants is one of the most successful criteria used by the pharmaceutical industry in finding new therapeutic agents for the various fields of biomedicine (Cox *et al*., 1994). Some outstanding medicinal drugs which have been developed from the ethnomedicinal uses of plants include: vinblastine and vincristine from *Catharanthus roseus* (the periwinkle) used for treating acute lymphoma, acute leukaemias etc. reserpine from *Rauwolfia serpentina* (Indian snake root) used for treating hypertension; aspirin from *Salix purpurea* (willow) used for treating inflammation, pain and thrombosis and quinine from *Cinchona pubescens* (cinchona) used for treating malaria (Lad, 2006). Today about 80% of the world's population rely predominantly on plants and plant extracts for healthcare (Setzer *et al*., 2006). In addition, of the top 150 proprietary drugs used in the United States of America (USA), 57% contain at least one major active compound currently or once derived from plants (Grifo *et al*., 1997). According to data released by the World Health Organization (WHO, 2003), ethnomedicine has maintained its popularity in all regions of the developing world and its use is rapidly expanding in the industrialized countries. For example, in China, traditional herbal preparation account for 30–50 % of the total medicinal consumption. In Ghana, Mali, Nigeria and Zambia, the first line treatment for 60 % of children with malaria is the use of herbal medicine. In San Francisco, London and South Africa, 70% of people living with HIV/AIDS

use traditional medicine. Today the annual global market for herbal medicine stands at over US $60 billion (WHO, 2003).

Contemporary events have grossly increased the demands placed on traditional medicine in Nigeria. These include the emergence of new types of diseases, the resurgence of old diseases, the appearance of drug-resistant strains of disease-agents, and the current crisis of western-type healthcare delivery, which makes it unable to provide sufficient care in urban Nigeria and virtually no care in most remote, rural communities (Izugbara, *et al*., 2004). Consequently there is need for more research on medicinal plants in order to meet these demands. This research work focuses on a plant commonly used in Nigeria for various ailments – *Cymbopogon citratus* (lemongrass).

Lemongrass is an aromatic grass belonging to the family Poaceae and the genus *Cymbopogon*. Poaceae, is the grass family, which is a very large cosmopolitan family consisting of about 50 to 60 tribes, 660 genera and 9000 species throughout the world (Olorode, 1984; Hutchinson *et al*., 1972). Cymbopogon is a genus of about 55 species, which are indigenous to tropical and semi-tropical areas of Asia and are cultivated in South and Central America, Africa and other tropical countries. The name Cymbopogon is derived from the Greek words “kymbe” (boat) and “pogon” (beard), referring to the flower spike arrangement (Plants database, 2003).

Lemongrass is well known for its oil and it is one of the world's best known essential oils.

There are two main types of lemongrass namely East Indian and West Indian. The East Indian lemongrass oil is obtained from *Cymbopogon flexuosus* Stapf and is the genuine oil of commercial importance. The West Indian oil is extracted from *Cymbopogon citratus* (DC) Stapf that is mainly cultivated in Central and South America and also known in parts of Africa, South East Asia and the Indian Ocean Islands. The name lemongrass has been given because of its

typical strong lemon - like odour, which is due to the high citral content. The two oils were formerly the main source of natural citral. The essential oil are used in perfumery, cosmetic and pharmaceutical preparations. A third species, *Cympobogon pendulus* (Nees ex Steud) Wats has been recently distilled in India to a limited extent. This grass also contains 70 - 80% citral. However it is more resilient than the two previous species