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Phytochemical Screening, Quantification and the Anti-Inflammatory Activity of the Methanolic Extract of *Ocimum gratissimum* Leaves

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ABSTRACT

Ocimum gratissimum belongs to the family Lamiaceae. Folkore medicine claims its use in anti- inflammatory condition. The aim of this work is to extract, analyze and quantify the phytoactive constituents and evaluate the anti - inflammatory effect. Extraction of the plant material was done in aqueous by maceration and then successively extracted in hexane, chloroform and methanol by sohxlet apparatus. Preliminary phytochemical screening was carried out to determine the active constituents in different solvent extract. Phytochemical quantification was done in the crude powder to determine the amount of saponins, tannins, flavonoids and alkaloids. Anti-inflammatory activities of the methanolic extract of *Ocimum gratissimum* was evaluated in the egg albumin -induced rat paw oedema. The phytochemical screening shows the presence of flavonoids in all solvent extracts and some selective secondary metabolites in other solvent extract. The crude powder shows highest amount of saponins and moderate amounts of tannins, alkaloids and flavonoids. The methanolic extract at 100 - 400 mg/kg demonstrated a dose dependent and significant inhibition (p<0.05) of oedema in the egg albumin - induced oedema in rats. This research contributed to the knowledge of the phytochemicals present and the anti-inflammatory activity of *Ocimum gratissimum* leaves.

Keywords: Anti-inflammatory, Ocimum gratissimum, phytovhemicals, egg albumin

INTRODUCTION

Medicinal plants have been of great important to the health of individual and communities. The medicinal value of these plants had some chemical active substances that produce a definite physiological action on the human body $\lceil 1 \rceil$. Nature has served as a rich repository of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably of plant origin [2]. Herbal medicine, based on their traditional uses in the form of powders, liquids or mixtures, has been the basis of treatment for various ailments in since ancient times [3]. Accordingto World Health Organization (WHO) traditional medicines was relied upon by 65-80% of the World's population for their primary health care needs [4]. Moreover, emergence of multiple drug resistant strain of microorganisms due to indiscriminate use of antibiotics to treat infectious diseases has generated a renewed interest in herbal medicine [5]. Inflammation was a normal protective response to tissue injury that was caused by physical trauma, noxious chemicals or microbiological agents [6]. The practice of traditional medicine, which was deep rooted in rural areas, continues unabated alongside conventional medicine because of ease of availability, inaccessibility of health centers and also due to social cultural factors [7]. Western style healthcare provided by the government is often not readily available and many regions remain completely underserved. Consequently, most communities still use herbal remedies as readily and cheaply available alternatives [8]. Ocimmum. gratissimum is an aromatic shrub from Asia and Africa. It is a herbaceous plant which belongs to the Labiatae family [9]. The plant is indigenous to tropical areas especially India and it is also in West and East Africa. The plant was found throughout the tropics and subtropics and its greatest variability occurs in tropical Africa and India [10]. In Nigeria Yoruba language, it was known as Efirinnla, Nchannwu in Ibo, Bunsurudaji in Hausa, Ireru in Ebira, Ebavbokho in Benin, ufuo-yibo in Urhobo and ntion in Efik[11] Ocimmum. gratissimum has been used extensively in the traditional system of medicine in many countries. In the Northeast of Brazil, it was used for medicinal, condiment and culinary purpose. The flowers and the leaves of this plant were rich in essential oils so it was used in preparation of teas and infusion [12]. Ocimmum gratissimum was commonly known as fever leaf but was divided into different native names in different part of the country [11]. It was used in

the treatment of epilepsy in the coastal area of Nigeria [13], High fever [14], and Diarrhea [14, 15]. The plant was also used to treat typhoid fever and diabetes [16]. Researches has shown that *Ocimmum gratissimum* posses anti-fertility effects in male mice [16]. *Ocimuum* gratissimum methanolic extracts showed a hepatoprotective effect [17]. Leaf extract of *O. gratissimum* showed antidiabetic properties in streptozocin-induced in diabetic rats [18], aphrodisiac activity [19], antibacterial, antifungal, antimicrobial, anthelmintic, in vitro anti-dermatophytic agent among other medicinal benefits [20,21]. *Ocimmum. gratissimum* leaves inhibits tumor growth and angiogenesis by affecting tumor cell proliferation, migration, morphogenesis, stromal apoptosis and induction of inducible cyclooxygenase (COX-2) [22]. This study aims to extract, analyze and quantify the phytoactive constituents and evaluate the anti - inflammatory effect.

MATERIALS AND METHODS

Plants Material

Dried leaves of *Ocimmum gratissimum* were ground into powder and store in air tight container prior to extraction. Chemicals and Reagents

Laboratory grade solvents: n- hexane, Chloroform, Ethanol, Methanol, Petroleum Ether, Diethyl ether, Ethyl acetate, Ethanol, and ether.

Preparation of the Extract

The dried material was crushed into powder, then, 100g of the powder was weighed into an empty beaker, and then soaked in 500mls of distilled water and then shaken for 48 hours. The extract was sieved and the juice was filtered using a clean white cotton cloth. The filtrate was put into cleaned and dried beakers and were placed in the water bath using retort stand and evaporated at 80°C and then dried in the oven at 40c. The gummy extract was put into already weighed small plastic dish and weighed in an electronic sensitive weighing balance. The percentage yield was calculated and then stored in the refrigerator for further studies.

Successive solvelt extraction using laboratory grade N-hexane, Chloroform and Methanol was carried out in a solvelt apparatus. Each solvent extract was pooled together, concentrated over the water bath at 40°C and dried in the oven. % yield of extraction was calculated as follows;

Weight of empty beaker +extract -weight of empty beaker $\times 100$ Weight of dried powder

Phytochemical Screening

The standard described by Trease and Evans (1996) was applied in this phytochemical screening of the individual constituents of the extract [22]. These compounds included: tannins, phlobatannins, saponins, terpenoids, flavonoids, alkaloids and reducing sugar.

Phytochemical quantification Quantification of total tannins

1 0g of the sample powder was weighed in a 200ml beaker using a weighing balance and there after macerated with 100ml of distilled water, rotated on a rotator for 20hours and left to stand for 24 hours. The maceration was filtered using small size Whatman No. I filter paper and the filtrate dried in a hot air oven at 70°C. The dried extract was weighed and recorded. The residue was dissolved with 5mls of distilled water and then extracted repeatedly with 20ml of petroleum ether while shaking at 15 minutes intervals for 1 hour. This purification process was repeated and there after tannins precipitated with 7% potassium dichromate. The precipitate was weighed to determine the weight of total tannins.

Quantification of total alkaloids

10g of the sample powder was weighed using a sensitive weighing balance and macerated in 100ml of distilled water with continuous shaking on a laboratory rotator at a speed of 200 rates per minute for 16 hours and then left to stand for 24 hours. The maceration was filtered and then washed through the filter paper with 2 successive portions of 100ml of distilled water. The filtrate and the filtered maceration washings was bulked together and the volume noted and then transferred to a beaker of known weight, evaporated to dryness at a temperature of 5° C. The acidified mixture was concentrated to half its original volume with a water bath at a temperature of 75° C, filtered and the filtrate basified with concentrated ammonia and centrifuged using a centrifuging machine model 800 at 1000 rates per minute for 30 minutes. The obtained solid was dissolved in 10ml of 2% aqueous acetic acid solution, basified with minimum concentrated ammonia until a precipitate was seen. The mixture was extracted with 2 successive 20ml portions of chloroform with vigorous shaking once every 15 minutes for 1 hour and the extraction repeated. The aqueous portions was collected in a beaker of known weight and evaporated to dryness in a hot air oven at 75° C. The weight of the gummy extract was determined using a sensitive balance.

Quantification of total saponin

10g of the sample powder was weighed in a 200ml beaker and macerated with 100ml of distilled water and rotated on a laboratory rotator for 15 hours and was left to stand for 24 hours. The maceration was filtered using

Whatman No. l small size filter paper and the filtrate reduced to 40ml over a water bath at about 90°C. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously every 15 minute for 1 hour. The aqueous layer was recovered while the ether layer discarded. The purification process was repeated and then, 60ml of n-butanol added to extract repeatedly. The combined n-butanol extract was washed twice with 10ml of 5% aqueous NaCl and the remaining solution heated in the hot air oven at 70°C. The residue was weighed to determine the quantity of saponins present.

Quantification of total flavonoids (Barbone)

10g of the sample powder was weighed using a sensitive balance and 100ml of distilled water shall be added. The mixture will be shaken continuously for 12 hours on a laboratory rotator at a speed of 100 rates per minute and then left to stand for 24 hours. The mixture was filtered using Whatman No.1 small size filter paper and then dried in the hot air oven for 20 hours. The weight of the extract was determined. The extract was re-extracted twice with 20ml portions of ethyl acetate and thereafter extracted with 10ml of amyl alcohol. The ethyl acetate was dried in a hot air oven at 70°C and weighed to determine the amount of flavonoids present.

Evaluation of anti-inflammatory activities by the egg- albumin induced rat paw oedema

Fresh raw egg-albumin was used as an in vivo model to induce acute inflammation. Rats were divided into five groups (n=5) and pretreated as follows; group 1 received 10ml/kg normal saline (control group), groups 2-4 received 100, 200 and 400 mg/kg of methanolic extract of *Ocimmum gratissimum* leaves, respectively, while group 5 received 10mg/kg indomethacin All treatments were administered orally. Oedema was induced 30 min later in all the rats by sub-plantar injection of 0.1 ml of raw egg albumin to the left hind paw. Oedema formation was taken as increase in paw circumference measured by wrapping a white cotton thread around the injected paw. Initial paw sizes were taken before injection of egg albumin, while subsequent measurements were taken at an interval of 30 min for a total of 120 min. Results were expressed as mean suppression of inflammation compared as with the preegg albumin injection value for each rat.

Statistical Analysis of Data

All data generated were presented as Mean \pm Standard Error of the Mean (SEM) ai1d statistical comparison of means was performed using student's t- test in Microsoft EXCEL and SPSS software. P < 0.05 was considered as statistically significant.

Table 1: Percent Yield of different solvent soxhlet extraction of Ocimum gratissimum leaves.			
Extract	Percentage yield		
Hexane	3.8%		
Chloroform	3.2%		
Methanol	7.7%		

RESULTS

Table 2: Preliminary Phytochemical Test for Different Solvent Extract of Ocimmum gratissimum leaves.

Phytochemical constituents	Aqueous	Hexane	Chloroform	Methanol
Alkaloids	+	-	-	+
Flavonoids	+	+	+	+
Saponins	+	-	-	+
Tannins	+	-	-	+
Reducing sugars	-	-	+	+
Terpenoids	+	+	+	-
Steroids	-	+	+	+
Phlobatannins	+	-	-	-

+=Presence of constituents; - = Not detected

Table 3: Percent Yield of Quantified Phytochemical Constituent in crude powder of Ocimmum gratissimum leaves.Phytochemical constituentsPercentage Yield in Crude Leaf

Flavonoids	9%
Alkaloids	22%
Tannins	18%
Saponins	62%

Table 4: Anti-inflammatory effect of *Ocimmum gratissimum* methanolic extract (OGME) on egg albumin -induced oedema in rats.

Treatment	Dosage	Paw circumference (cm)				
	(mg/kg, p.o)	0 min	30 min	60 min	90 min	120 min
Contro	10ml/kg	$2.14{\pm}~0.02$	2.74 ± 0.06	2.78 ± 0.09	2.58 ± 0.07	2.52 ± 0.1
OGME	100	2.14 ± 0.06	2.7 ± 0.04	2.56 ± 0.07	2.46 ± 0.04	2.36 ± 0.07
OGME	200	2.1 ± 0.04	2.52 ± 0.06	2.5 ± 0.05	2.42 ± 0.05	2.48 ± 0.05
OGME	400	2.18 ± 0.02	2.58 ± 0.07	2.5 ± 0.05	2.4 ± 0.04	2.32 ± 0.05
indomethacin	10	2.12 ± 0.06	2.68 ± 0.06	2.5 ± 0.03	2.48 ± 0.04	2.36 ± 0.07

Results are Mean \pm SEM (n=5)

Table 5: Difference paw circumference								
Treatment	Dosage	Difference in paw circumference (cm)				Difference in paw circumference (cm)		
	(mg/kg,p.o	30min	60min	90min	120min			
)							
Control	10ml/kg	0.6	0.64	0.44	0.38			
OGME	100	0.56(6.7)	0.42(34.4)	0.32(27.3)	0.22(42.1)			
OGME	200	0.42(30)	0.4(37.4)	0.32(27.3)	0.28(26.3)			
OGME	400	0.4(33.3)*	0.32(50)*	0.22(50)*	$0.14(63.2)^*$			
Indomethacin	10	0.56(6.7)	0.38(40.6)	0.36(18.2)	0.24(36.8)			

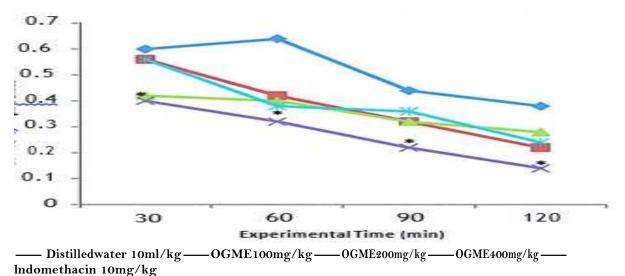


Fig 1: Effect of OGME on egg albumin-induced acute oedema in rats.

DISCUSSION

Ocimmum gratissimum has been a popular herb mainly used has a folk medicine, meaning that it is collected and used by lay persons to treat the common ailments [23]. Its uses have been attributed to the presence of essential oils and flavonoid constituents. Flavonoids are known anti-inflammatory constituents present in many plant extracts. In our solvent extraction, flavonoids were present in all the solvent extracts of Ocimmum gralissimum

(Table 1). Other secondary metabolites found in the different solvent extracts are alkaloids, tannins, saponins and steroids. Medicinal properties of plants are due largely to their phytochemical compositions $\lceil 24, 25 \rceil$ Steroids was known to exert their anti-inflammatory effect by inhibiting phospholipase A2, a key enzyme of arachidonic acid metabolism, thereby stopping prostaglandin synthesis [26]. Some plant steroids have also been shown to stabilize lysozomal membranes there by inhibiting the release of pro-inflammatory mediators [27]. Alkaloids and flavonoids have also been shown to possess anti-inflammatory activity by inhibiting the action of arachidonic acid metabolism via the cyclooxygenase and 5-lipoxygenase pathways [28]. Flavonoids also inhibit inflammation by lysozomal membrane stabilization and also by inhibiting migration of leucocytes to the site of inflammatory stimulus [29]. The egg-albumin induced inflammatory reaction has been shown in two phases. The early phase, which begins at 30 minutes after administration of the albumin results from the release of histamine, serotonin and bradykinin; while the later phase (1h after irritant administration) is associated with the release of mediators such as prostaglandins [30, 31]. The result of the anti-inflammatory effect of Ocimmum gratissimum shown in table 4.4 indicated that the methanolic extract exhibited significant inhibition of acute edema induced by egg albumen. The oedema peaked at I hr and gradually declined to 2hrs. The methanolic extract of Ocimmum gratissimum extract produces a pronounced dose-dependent anti-inflammatory effect in both phases of albumin induced oedema. From the above results, it can be suggested that ant-inflammatory effect of the plant extract on albumin induced oedema may be related to inhibition of the release of mediators such as prostaglandins (PGE2 and PG12) which have been implicated as mediators of the inflammatory and pain responses.

CONCLUSION

The results of the present study have shown that the leaf of *Ocimmum gratissimum* is rich in secondary metabolites -sapomns, flavonoids, tannins and alkaloids and that the methanolic extract possesses significant antiinflammatory activity on acute inflammation in rats thus, justifying its use by traditional medicine practitioners in the management of disease conditions associated with inflammation.

Recommendation

Further studies on anti-inflammatory activities in hexane and chloroform fraction. Purification and characterization of the potent anti-inflammatory compounds.

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