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Phytochemical Analysis, Elemental Determination and Some *in vitro* Antibacterial Activity of *Ocimum basilicum* L. Leaf Extracts

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Abstract: *Ocimum basilicum* Linn (Lamiaceae) leaf extract was investigated for its chemical and elemental contents as well as its antibiotic effects. The aqueous soxhlet extract, which is normally used in folkloric medicine and the chloroform, n-butanol and ethyl acetate fractions were subjected to phytochemical screenings. Phytochemical analysis of the aqueous leaf extract revealed that the concentration of saponin and alkaloids were high, flavonoids, terpenes and steroids were present in medium quantity, while traces of tannins and carbohydrates were also present in the aqueous extract. The phytochemical investigation of the various solvent fractions showed that all the three fractions contain carbohydrates, terpenes and steroids, tannins and flavonoids. In addition, the chloroform and n-butanol fractions contain cardiac glycosides. The result showed high concentration of Na, K and SO_4, while those present in trace quantity include Mg, Mn, Zn, Cu and Pb. The extracts of *O. basilicum* were found to possess *in vitro* antibiotic activity against *Staphylococcus aureus*, streptococcus species, salmonella species, shigella species, *Pseudomonas aureginosa* at high doses. In conclusion, this study has shown that *Ocimum basilicum* contain chemical elements that can be pharmacologically useful as well as possess some antibacterial properties.

Key words: Phytochemical, elemental, *Ocimum basilicum* extracts, antibacterial

INTRODUCTION

Ocimum basilicum L. contains eugenol that is responsible for its clove scents. It is the most widely used of the *Ocimum* species for its potential medicinal properties. The dried leaves of *O. basilicum* contain 0.20-1% essential oils. Literature however revealed that about 45 componnds are fonnd in volatile oils of this plant with the major compounds being linalool, eugenol, methylchaviol, methylcinnamol, linolen, olimene, pinene, cineol, anethol, estragol, thymol, citral and camphor (Mirheidar, 1990; Wang *et al*., 2003; Grayer *et al*., 1996). Quantitative analysis of the various parts of the plant showed that *Ocimum basilicum* leaf contain the following in parts per million (ppm): Caffeic acid (19,000), P-Coumaric acid (760), Myrcene (2-80), Tryptophan (2210) and Rutin while the essential oil contain 1,8-Cineole (1.6-8%), P-Cymene (<0.1%), Limonene (<1.0%), Linalool (1.1-65.4%), Methylchaviol (13.5-87.2%), Methylcynamate (0-11.2%), Myrcene (0.1-2%), Alpha Pinene (0.2-0.4%), B-Pinene (<1.0%) and Alpha-Terpinene (0.03-0.2%). Analysis of the whole plant indicated the presence of 1,8 Cineole (776 ppm), P-Cymene (1-16 ppm), Limonene (2-934 ppm), Linalool (30-300 ppm), Methylchaviol (238-8,780 ppm), Methylcinnamate (1-2800 ppm), Alpha-Pinene (2-180 ppm), B-Pinene (3-160 ppm), Safrole (60,400 ppm) and Quercetin (van Duong, 1993; Beckstrom-Sternberg *et al*., 1994; McCorkle *et al*., 1996).

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Report indicated that the fixed oil of *O. basilicum* possesses significant anti-inflammatory activity against carageenan and different other mediator induced paw edema in rats; it also inhibited arachidonic acid and leukotriene induced paw edema; has significant inhibitory effect on castor oil-induced diarrhea in rats (Singh, 1999). The essential oil, has been shown *in vitro* to have antibacterial activity against *Staphylococcus aureus*, *Salmonella enteritidis* and *Escherichia coli*, antiseptic activity against *Proteus vulgaris*, *Bacillus subtilis* and *Salmonella paratyphi* and antifungal activity against *Candida albicans*, *Penicillium notatum* and *Microsporeum gyseum*. The oil has also been shown to repel insects and have larvicidal activity against houseflies, blue bottle flies and mosquitoes (Beckstrom-Sternberg *et al*., 1994; McCorkle *et al*., 1996).

This study was therefore carried out to identify the chemical and elemental constituents of *Ocimum basilicum* leaves as basis for validation of its pharmacological activities.

MATERIALS AND METHODS

Sample Collection and Identification

Leaves of *O. basilicum* without stalk were collected and used in November 2006 from the botanical garden belonging to Professor A.B. Anaso, University of Maiduguri, Borno State, Nigeria. A plant taxonomist in the Department of Biological Sciences, University of Maiduguri, Maiduguri, Nigeria, was responsible for its identification. A voucher specimen was deposited in the Department of Veterinary Physiology and Pharmacology Research Laboratory, University of Maiduguri. The sample was air dried in the shade and ground in a grinder to a fine powder and stored in a plastic container at 4°C, until required.

Plant Extraction

- **Aqueous extract:** The dried powdered plant materials (5.0 g) was exhaustively water extracted using Soxhlet extractor. The crude extract was concentrated *in vacuo*, properly labeled and stored in refrigerator at 4°C until used (Trease and Evans, 1989).
- **Fractionation of the aqueous extract:** The crude aqueous extract obtained was suspended in cool distilled water and then filtered using Whatman No. 1 filter paper. The filtrate was thereafter fractionated successively with chloroform, ethyl acetate and normal butanol (Fig. 1). The fractionation with the organic solvents which are of different polarity was done until the organic layer were visibly clear to get chloroform, ethyl acetate and n-butanol soluble fractions and residual aqueous fraction, in sequence as described by Cho *et al*. (2003) and Motohashi *et al*. (2004).

Identification of the Chemical Constituents in the Extracts of *O. basilicum*

The aqueous extracts and the solvent fractions of the plant were subjected to qualitative chemical screening for the identification of the various classes of chemical constituents using the method described by Trease and Evans (1989), Clarke (1975) and Odebiyi and Sofowora (1978).

Determination of Elemental Content of *O. basilicum* Leaf

Air-dried sample (15 g) of *O. basilicum* was put in a labeled crucible and heated in a hotspot furnace at 550°C for 3 h. The sample was removed and cooled in a dessicator. Half (0.5) gram of the ashed sample was digested in a 250 mL beaker with 20 mL of 2 M nitric acid and 10 mL of 35% hydrogen peroxide and heated on a hot plate in a fume cupboard until a clear digest was obtained. The content was then filtered after cooling and deionized water added and made up to 100 mL in a

volumetric flask for elemental analysis using sp-9-single beam atomic absorption spectrophotometer (Philip/Pye Unicam Ltd., England). The elemental concentrations were determined by a standard calibration curve method (Sunderman, 1973; Kolthoff and Elving, 1976).

Effect of the Extract on Bacterial Activity

The disc-diffusion method as described by the National Committee of Clinical Laboratory Standards (1993) was used to determine the antibacterial activity of the aqueous leaf extract of *O. basilicum*. Discs containing different concentrations of dissolved extracts was prepared with sterilized filter papers (Whatman No. 1; 6 mm in diameter) soaked in different beakers containing 100, 200, 400 and 700 mg mL^{-1} of the extract. The discs were dried at 50°C. Overnight cultures of each bacterial isolates were diluted using sterile normal saline to give an inoculum size of about 10^6 cfu mL^{-1}. The inocula were spread on the surface of dried nutrient agar plates with cotton wool swabs, which have been dipped in the diluted suspension of the organisms. The plates were incubated at 37°C for 30 min before the discs were applied aseptically. The treated plates were incubated at 37°C for 48 h. The same procedure was carried out using oxytetracycline (10 mg mL^{-1}) as the positive control. Plates without the antibiotic or extract discs were set up as the negative control. The zone of inhibition above 6 mm diameter of each isolate was used as a measure of susceptibility to the extract and this was compared to that of the standard antibiotic.

RESULTS

The extract gave a yield of 33.12% w/w, had an ox-blood colour and readily dissolves in water. The result of the phytochemical analysis of the aqueous leave extract of *O. basilicum* showed that saponin and alkaloids are the most abundant compounds present in the extract while flavonoids, cardiac glycosides, terpenes and steroids were present in medium quantity and tannins and carbohydrates occurred in minute quantities (Table 1).

The ethyl acetate soluble fraction of the extract occurred more in quantity, followed by the n-butanol fraction and then the chloroform fraction (Fig. 1). The phytochemical investigation of the various solvent fractions showed that all the three fractions contain carbohydrates, terpenes and steroids, tannins and flavonoids. In addition, the chloroform and n-butanol fractions contain cardiac glycosides (Table 2).

The elemental analysis showed high concentration of Na, K and SO_4 (930.0, 102.12 and 100.0 ppm), respectively. Ca, Cl, Ni and Fe were present at 31.33, 19.0, 12.80 and 11.80 ppm, respectively, while those present at minute quantity include Mg, Mn, Zn, Cu and Pb at 1.79, 0.80, 0.65, 0.44 and 0.19 ppm, respectively (Table 3).

Table 1: The phytochemical analysis of *O. basilicum* aqueous leaf extract

Phytochemical constituents	Inference
Carbohydrate	+
Tannin	+
Antraquinone	-
Saponin	+++
Cardiac glycosides	++
Terpenes and steroids	++
Flavonoid	++
Alkaloids	+++

-: Not detected, +: Present in low concentration, ++: Present in moderate concentration, +++: Present in high concentration

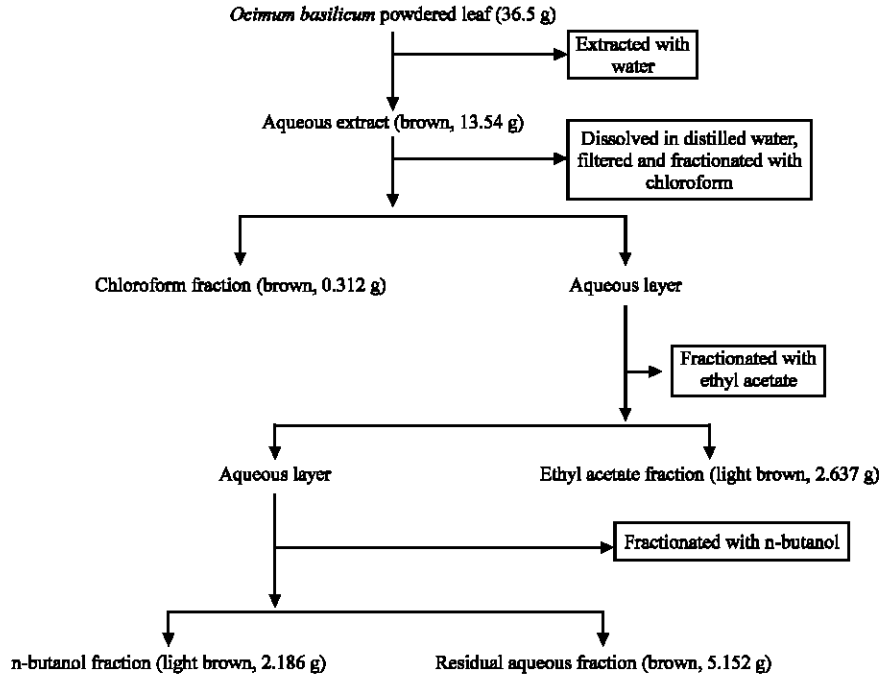

Fig. 1: Schematic diagram of extraction and fractionation of *O. basilicum* leaf extract into organic solvent fractions

Table 2: The phytochemistry of organic solvent fractions of aqueous extract of *Ocimum basilicum* leaf

Phytochemical constituents	Inference		
	Chloroform	Ethyl acetate	n-butanol
Carbohydrate	+	+	+
Tannin	+	+	+
Anthraquinone	-	-	-
Saponin	-	-	-
Cardiac glycosides	++	-	++
Terpenes and steroids	+	+	+
Flavonoid	+	+	+
Alkaloids	-	-	-

-: Not detected, +: Present in low concentration, ++: Present in moderate concentration, +++: Present in high concentration

Table 3: Elemental contents of *O. basilicum* leaf

Elements	Concentration (ppm)
Iron (Fe)	11.8031
Calcium (Ca)	31.3331
Magnesium (Mg)	1.7887
Manganese (Mn)	0.8000
Lead (Pb)	0.1891
Copper (Cu)	0.4448
Zinc (Zn)	0.6470
Potassium (K)	930.00
Sodium (Na)	102.12
Nickel (Ni)	12.80
Chlorine (Cl)	19.00
Sulphate (SO_4)	100.00

Table 4: Antibacterial activity of *O. basilicum aqueous leaf* extract

	Antibacterial activity (cm)					
Concentrations (mg mL^{-1})	*Staph. aureus*	Strep. spp.	Salmonella spp.	*E. coli*	Shigella spp.	*Pseudomonas aureginosa*
Extract (100)	R	R	R	R	R	R
Extract (200)	R	R	R	R	R	R
Extract (400)	R	R	0.8	R	0.7	R
Extract (700)	0.8	1.0	1.2	R	0.8	0.7
Tetracycline (250)	2.2	2.5	2.0	2.8	3.0	1.8

R: Resistance to the organism

Table 5: Antibacterial activity of *O. basilicum aqueous leaf* extract

	Antibacterial activity (cm)					
Concentrations (mg mL^{-1})	*Staph. aureus*	Strep. spp.	Salmonella spp.	*E. coli*	Shigella spp.	*Pseudomonas aureginosa*
Chloroform (200)	R	R	R	R	R	R
Chloroform (400)	R	R	R	R	R	R
Ethyl acetate (200)	1.3	1.2	R	R	R	R
Ethyl acetate (400)	1.7	1.5	R	R	R	R
N-butanol (200)	1.0	1.2	R	R	R	R
N-butanol (400)	0.9	1.2	R	R	R	R
Tetracycline (250)	2.2	2.5	2.0	2.8	3.0	1.8

R: Resistance to the organism

The aqueous extract inhibited the growth of *Staphylococcus aureus*, streptococcus species, salmonella species, shigella species and *Pseudomonas aureginosa* at 700 mg mL^{-1}. There was also inhibition of salmonella and shigella species at 400 mg mL^{-1} extract concentration. The standard drug, tetracycline, at 250 mg mL^{-1} inhibited the growth of all the species tested. Comparatively, the diameter of zone of inhibition by the extract was relatively doubled by that produced by the standard drug (Table 4).

The ethyl acetate and n-butanol fractions produced inhibition of *Staphylococcus aureus* and streptococcus species at 200 and 400 mg mL^{-1} only. However the ethyl acetate fraction produced better results than the n-butanol faction. The chloroform fraction had no effect on the organisms tested, while Tetracycline (standard drug) inhibited the growth of all the organisms tested at 250 mg kg^{-1} (Table 5).

DISCUSSION

The phytochemical investigations and elemental analysis of the aqueous leaf extract of *O. basilicum* indicated the presence of pharmacologically useful classes of compounds. Tannins was reported to possess physiological astringent properties, which hasten wound healing and ameliorate inflamed mucus membrane (Tyler *et al*., 1988). It also has hemostatic properties (Awosika, 1991). Saponin has expectorant action, which is very useful in the management of upper respiratory tract inflammation; saponin present in plants is cardiotonic in nature (Finar, 1989; Trease and Evans, 1989). Saponin is also reported to have antidiabetic properties (Kamel, 1991). Alkaloids are reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pains, develop resistance against diseases and endurance against stress (Gupta, 1994).

The presence of trace elements showed that the extract might usefully influence various body functions. These elements are used extensively in chemotherapy and are essential in human and animal health (Khan, 1996; Ogugbuaja *et al*., 1997; Moses *et al*., 2002). The presence of high concentration of some elements seen in this analysis of *O. basilicum* may be due to the topography, soil-water-plant exchange complex and evapotranspiration of the environment (Adonm *et al*., 1998). Some classes of

chemical compounds and elements found in the extract have been known to exert pharmacological effects while others are capable of protecting the active ingredients in the herb from decomposing either chemically or physiologically (Abdulrahman and Onyeyili, 2001).

The antibacterial effect seen in this experiment strengthens the basis for the folkloric report that the extract is used in the treatment of diarrhea, dysentery and wound healing. The organisms tested have been implicated in diarrhea, dysentery and wound infections. The antibacterial property could be due to flavonoids and tannins contents of the extract, both of which are known to possess appreciable antimicrobial activities (Narayana *et al*., 2001). The aqueous crude extract has been shown to contain carbohydrate, saponin, steroid and cardiac glycosides. Carbohydrate could facilitate the growth of bacterial organisms and therefore may antagonize antibacterial activity of the active principle which may account for activity seen ouly when high concentrations of the extract were used in this study.

In conclusion therefore, the result of the present study has provided supportive scientific evidence that the leaf extract of *Ocimum basilicum* possess antibacterial activity as well as possess some chemical principles that may be pharmacologically important.

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