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(RESEARCH ARTICLE)



Phytochemical screening, *in vitro* antibacterial and partial TLC purification of different solvents extracts of *Ocimum basilicum* L

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Abstract

Ocimum basilicum L is commonly used in Nigeria folk medicine for healing various diseases. In a continuous bid to explore new biocompatible antimicrobial agents with the least associated side effects, different solvents extract of *O. basilicum* were challenged with common pathogenic microorganisms at varying concentrations. The phytochemical screening, antimicrobial activity and the partial TLC purification of the different solvents extract were performed using standard procedures. The phytochemical analysis revealed the presence of various pharmaceutically active secondary metabolites like phenolic, flavonoids, carbohydrates, glycosides, tannins among others. Using the zone of inhibition as inhibitory parameter, the crude aqueous extract exhibited the best antibacterial activity among all the other solvent extracts but was lower than the standard drug ciprofloxacin which exhibited a near 100% activity for all microorganism screened. In conclusion, the plant leaves possesses various potentially active secondary metabolites which would help in developing pharmaceuticals especially antimicrobial drugs.

Keywords: *Ocimum basilicum*; Phytochemical; Antimicrobial activity; TLC

1. Introduction

Since decades, plants and their parts are well known for their bioactive constituents responsible for the therapeutic effects [1-3]. Medicinal plants are useful for healing and as well for curing human diseases because of the presence of phytochemical constituents [4]. They are rich in phytochemicals which offer defense mechanism and protection from various diseases [5]. As a result of the increase in the incidence of side effects and the resistance that pathogenic microorganisms build against several antibiotics, research on medicinal plants has attracted a lot of attentions globally [6-8], hence, the need to screen for potent therapeutic agents from plants origin which are easily accessible, cheaper and have bearable side effects.

Ocimum basilicum L. commonly called “king of herbs” belongs to the family Lamiaceae [9]. It is an annual plant usually producing white-purple flowers [10]. It is a culinary herb consumed in high quantity due to the characteristic flavors it imparts. The plant is found in many parts of the world especially in the tropical regions of Asia, Africa and Central and South America [11-12]. It contains many phytochemical compounds which manifests various health benefits [13]. Though much antibacterial and phytochemical studies has been conducted on *Ocimum species*, the present work has been designed to challenge different solvents extract of the plant against common pathogens with a view to screening for its antimicrobial activity, validating its traditional use and attempt to partially purify the different solvents extract using TLC.

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2. Material and methods

2.1. Plant materials collection, preparation and identification

The fresh plant materials of *Ocimum basilicum* (locally called Daddoya in Hausa) was collected at Baha area of Yauri town, Kebbi State in May, 2016 by hand picking. The leaves were washed under running tap water to remove earthy impurities. They were placed in sterile containers and transported to the Department of Pharmaceutical Microbiology Laboratory, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto Nigeria for analysis. Botanical identification and authentication of the sample was done by a plant Taxonomist of the Department of Botany, Faculty of Science, Usmanu Danfodiyo University Sokoto Nigeria where a herbarium specimen was deposited and a voucher number issued. They were then dried under shade for three weeks, powdered with the aid of a clean mechanical grinder and stored in an air tight glass container until use.

2.2. Chemicals

Methanol (Merck India), acetone (Merck India), n – hexane (Sigma Aldrich), ethyl acetate (Sigma Aldrich), and other chemicals and reagents used for the research were all of analytical grade.

2.3. Soxhlet extraction

The powdered leaves were extracted using a Soxhlet apparatus with analytical grade n – hexane. The cycles were repeated until complete defatting was obtained. The crude extract was then dried at room temperature to constant weight. This was the crude n - hexane leaves extract (CNE). The same procedure was repeated separately for three fractions, namely ethyl acetate, methanol and water fractions to obtain the CEE, CME and CAE respectively. All the fractions were stored at 4 °C till use.

2.4. Phytochemical analysis

Simple chemical tests to detect the presence of carbohydrates, proteins and secondary metabolites in the powdered *O. basilicum* leaves were done in accordance with standard methods [14-16].

2.5. Collection of the microorganisms

Standard laboratory strains of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Proteus spp.* were obtained from the Department of Pharmaceutical Microbiology, Usmanu Danfodiyo University Teaching Hospital Sokoto, Sokoto State Nigeria.

2.6. Confirmation of test organisms

Biochemical analysis methods as described by [17-19] were carried out on each of the test bacteria isolates for confirmation. The Bergey's Manual of Systematic Bacteriology [20] was used for species authentication.

2.7. Preparation of culture medium

The preparation of this medium was based on the manufacturer's specification and sterilization was by autoclaving at 121 °C.

2.8. Preparation of standard bacterial suspensions

The average number of viable, bacterial organisms per ml of the stock suspensions was determined by means of the surface viable counting technique [21]. About 10^8 - 10^9 colony forming units per ml were used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained [22].

2.9. Susceptibility test

The agar well diffusion method was used with slight modification [23]. The nutrient agar was prepared by dissolving accurately weighed 14 g of the agar into 500 cm³ of distilled water. The prepared agar was heated over a Bunsen burner to dissolve completely and it was dispensed in 20 cm³ of universal bottles, and then autoclaved at 121 °C for 20 minutes. It was allowed to cool and then distributed into sterilized Petri dishes (which have been sterilized at 170 °C for 1 hour) and allowed to set. After setting, the Petri dish was flooded with 1 cm³ of the standardized culture of various dilutions of the test bacteria and the excess was drained using a Pasteur pipette. Wells measuring 6 mm in diameter were bored

into the inoculated Petri dishes using a cork borer. The wells were sealed by a drop of melted nutrient agar and were filled with 0.05 cm³ each of 10 mg/ cm³, 20 mg/ cm³, 30 mg/ cm³ and 40 mg/ cm³ of the CNE, CEE, CME and CAE fractions respectively. The plates were allowed to stand for 2 hours for pre-diffusion and then incubated for 24 hours at 37 °C. The zone of inhibition was measured to the nearest millimeters using a metric rule. 10% Tween-40 (50 µL) and Ciprofloxacin (20 µg/mL) was used as the negative and positive control respectively. All readings were performed in triplicate.

2.10. TLC partial purification of the fractions of *O. basilicum*

TLC was performed on pre-coated TLC plates with silica gel F₂₅₄ (layer thickness 0.2 mm, E. Merck, Darmstadt, Germany). 8 cm³ of n-hexane and 2 cm³ of ethyl acetate were used as the solvent system. A line was drawn on the TLC plate to serve as the origin. A capillary tube was used to spot the n-hexane extract on the TLC plates and then placed in the solvent system. The distance travelled by the solvent was measured using a ruler. The same procedure was performed for the ethyl acetate and methanol fractions with ethyl acetate and n-hexane (8:2) and chloroform and methanol (5:1) were used as solvent systems respectively. To ensure accuracy of determination and also for compounds that are colorless, the TLC plate was viewed under ultraviolet (UV) light at 254 nm as well as inserting the TLC plate in iodine vapors for a few minutes.

3. Results and discussion

3.1. Percentage yield of the crude methanol extract and fractions

The percentage yield of the n-hexane (CNE), ethyl acetate (CEE), methanol (CME) and the aqueous (CAE) are presented in Table 1.

Table 1 Percentage yield of the different solvents used in extracting the *O. basilicum*

| Fractions | % Yield |
|-----------|---------|
| CNE | 25.51 |
| CEE | 2.77 |
| CME | 3.76 |
| CAE | 67.68 |

3.2. Phytochemical screening

Table 2 Preliminary phytochemical screening of the leaves of *O. basilicum*

| Phytochemicals | Test | Observation | Inference |
|--------------------------|----------------------|---|-----------|
| Saponin | Frothing | Foam was formed | + |
| Flavonoid | Shinoda's | Reddish colour was observed | + |
| | Alkaline reagents | Intense yellow colour was formed | + |
| Tannins | Ferric chloride | Blue black colour appeared | + |
| | Lead sub acetate | Coloured precipitate appeared | + |
| Anthraquinone | Borntrager's | No change was observed | - |
| Phenols | Ferric chloride | Bluish black colour appeared | + |
| Alkaloid | Mayer's | No precipitate formed | - |
| | Wagner's | No precipitate formed | - |
| | Dragendroff's | No precipitate formed | - |
| Carbohydrate | Molich's | Violet ring was formed at the junction | + |
| | Fehling's | Red precipitate was formed | + |
| Polyphenol/triterpenoids | Salkowski's | Appearance of golden yellow ring at the interface | + |
| | Libermann Burchard's | Formation of brown ring at the junction | + |
| Cardiac glycosides | Keller-Killiani's | No change was observed | - |

(+) = Presence of phytochemical; (-) = Absence of phytochemical

The result of the phytochemical analysis of the leaves of *O. basilicum* is presented in Table 2. The results of the phytochemical screening are similar with the results obtained by several researchers [24-26]. The presence of these phytochemicals is responsible for the diverse tradomedical application of the plant.

3.3. Antimicrobial screening

The result presented in Figure 1 reveals the *in vitro* susceptibility of the bacteria isolates to the crude fractions of *O. basilicum*. The results shows the mean \pm standard deviation of the inhibition zone (mm) for triplicate determination in the various agar plates of bacteria exposed to the extract fractions compared to the standard drug ciprofloxacin.

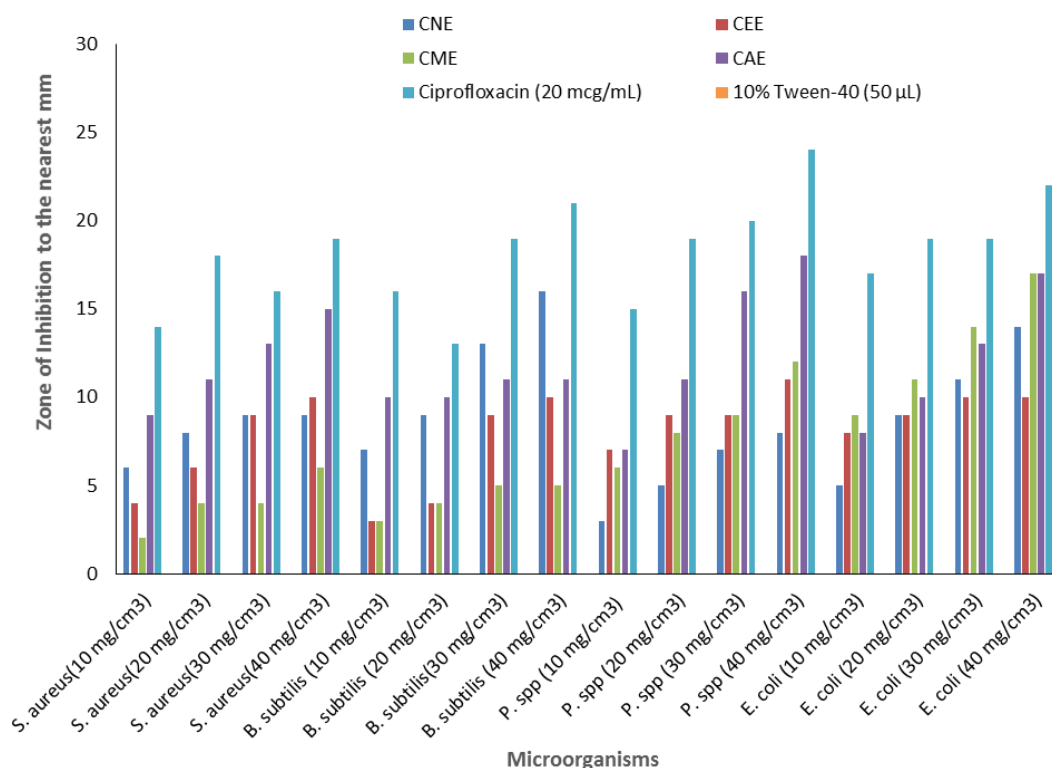


Figure 1 Antimicrobial activity (zone of inhibition) of *O. basilicum* crude extract fractions against various human pathogens (CNE = Crude n-hexane extract, CEE = crude ethyl acetate extract, CME = crude methanol extract, CAE = crude aqueous extract).

Natural antimicrobial compounds from plants are now been explored since they not only offer therapeutics influence but offer fewer side effects when compared to synthetic antimicrobial agents. The different solvent extract showed varying activity against the bacteria isolates investigated with the CAE showing stronger and broader spectrum of antimicrobial activity compared to other solvent extracts. Highest zone of inhibition of 18 mm was observed at 40 mg/ml concentration of the CAE against *P. spp* while the least activity was exhibited by the CME at 10 mg/ml with a zone of inhibition of 2 mm when challenge with *S. aureus*. While the 10% Tween - 40 solutions showed no activity, the ciprofloxacin exhibited a 100% or near 100%. The antimicrobial activity recorded for the different solvents extract are in agreement with the report of Adiguzel *et al.*, [27], Adamu *et al.*, [28], Adam and Omer, [29] who all reported the antibacterial activities of different extracts of *O. basilicum* leaves against several bacterial strains using different methods. The antimicrobial results also agrees with the findings of Cooper *et al.*, [30] who reported that the presence of more group of phytochemical diversity gives synergic effects in many biological applications. The disparity between the activities of the extracts and the standard antimicrobial drugs may be due to the mixtures of bioactive compounds present in the extracts compared to the pure compound contained in the standard antibiotics [31].

3.4. Partial purification of the fractions of *O. basilicum* using TLC

The result of the T.L.C partial purification of the CNE, CEE and CME fractions *O. basilicum* are presented tables 3 – 5

Table 3 TLC purification of the CNE extract

| Spot | R _f value |
|------|----------------------|
| A | 0.90 |
| B | 0.61 |
| C | 0.44 |
| D | 0.38 |
| E | 0.31 |
| F | 0.27 |
| G | 0.18 |
| H | 0.13 |

Solvent system: Ethyl acetate: Hexane (2:8, v/v)

Table 4 TLC purification of the CEE extract

| Spot | R _f value |
|------|----------------------|
| A | 0.94 |
| B | 0.83 |
| C | 0.75 |
| D | 0.60 |
| E | 0.54 |
| F | 0.19 |

Solvent system: Ethyl acetate: Hexane (8:2, v/v)

Table 5 TLC purification of the CME extract

| Spot | R _f value |
|------|----------------------|
| A | 0.96 |
| B | 0.90 |
| C | 0.64 |
| D | 0.46 |
| E | 0.34 |

Solvent system: Methanol: Chloroform (1:5 v/v)

The TLC chromatogram of the CNE, CEE and CME showed eight, five and five spots respectively when suitable solvent systems were employed. These results showed that the CNE, CEE and CME extracts mixtures has at least eight, five and five components respectively since some components may have such similar polarities that they appear under one spot after development.

4. Conclusion

The present study summarizes that *O. basilicum* is a good source of important secondary metabolites which are responsible for its diverse pharmacological application. The different solvent extracts showed promising broad spectrum antimicrobial activity and hence could be a potential source of natural antimicrobial agent. These results encourage researchers to do further in vitro and in vivo researches that will explore the role of bioactive constituents responsible for these activities as well as carry out studies at molecular level.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interests and are solely responsible for all the contents and writings presented in this research work.

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