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Phytochemical Screening of leaf extract of *Ocimum gratissimum* L. *Var.* Clocimum

Dr. Hanmant R Aglave
Principal, Shahir Annabhau sathe Mahavidyalaya. Mukhed, Maharashtra, India

Abstract
The ethanolic extracts of the leaves of *Ocimum gratissimum* L. *Var.* Clocimum were used in the present study. The plants were screened to evaluate the phytochemical screening of ethanolic extract of O.G. Phytochemical screening of the plants showed the presence of flavonoids, terpenoids, saponins and tannins. OG have alkaloids and saponins were present in appreciable amounts. Glycosides and phenols were present in moderate amounts while tannins, steroids, flavonoids, phlobatannins, and anthraquinones were present in minute quantities Cardenolides, terpenes were absent. The studies shows that the presence of flavonoids and tannins in all the plants is likely to be responsible for the free radical scavenging effects. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers.

Keywords: *Ocimum gratissimum*, phenols, alkaloids, flavinods, terpenes

Introduction
Phytochemicals are chemicals of plant origin [1]. Phytochemicals (from Greek *phyto*, meaning "plant") are chemicals produced by plants through primary or secondary metabolism [2, 3] They generally have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens, or predators[2]. Phytochemicals generally are regarded as research compounds rather than essential nutrients because proof of their possible health effects has not been established yet [4, 5]. Phytochemicals under research can be classified into major categories, such as carotenoids and polyphenols, which include phenolic acids, flavonoids, and stilbenes/lignans [5]. Flavonoids can be further divided into groups based on their similar chemical structure, such as anthocyanins, flavones, flavanones, and isoflavones, and flavanols [5].
Ocimum gratissimum L. (O.G.) is a herbaceous perennial plant commonly known as scent leaf, found in tropical Asia especially India. *Ocimum gratissimum* L. *Var.* Clocimum is a new hybrid strain of O.G developed by Sobti *et al* from Indian Institute of Integrative Medicine formerly Regional Research Laboratory, Jammu Tawi. It has been used extensively in the traditional system of medicine in many countries. It is used in the treatment of various diseases like cancer, antineoceptive, anti-inflammatory, anti-diahorreal, antibacterial, antifungal and as nephroprotective [15, 16, 17]. The essential oil of *Ocimum gratissimum* contains eugenol and shows some evidence of antibacterial activity [21, 22] The essential oil has potential for use as a food preservative and is toxic to *Leishmania* [23].
Various reports on phytochemicals present in ethanolic extract of O.G. showed the presence of carbohydrates, alkaloids, terpenoids, phenolics, tannins, flavonoids, anthraquinones, sterols, glycosides and saponins [20]. While the phytochemical analysis on both the fresh and dried leaves of the plant revealed the presence of terpenes, flavonoids, tannins, alkaloids, steroids, proteins, carbohydrates, fats and oils with the dried samples having higher concentrations [17]. The present study has been undertaken to evaluate the phytochemical screening of ethanolic extract of O.G.

Material and Methods
Plant collection and identification:
The plant of O.G. was collected from Kolar road, Bhopal, Madhya Pradesh, and plant was identified with the help of regional Floras (Figure 1).

Fig 1: *Ocimum gratissimum*

Dry leaf preparation
The leaves were obtained from a garden in Ado Ekiti, Nigeria. Leaves were sorted and gently rinsed. The leaves were then spread on paper inside a room for 5 days to dry and then ground using a blender.

Phytochemical screening
Leave powder was soaked in water for 24 h at room temperature and then filtered. Chemicals tests were carried out on the extract using standard procedure to identify the constituents as described by Sofowora [6], Trease and Evans [7] and Harborne [8]. Phytochemicals screened were: tannin, phlobatannin, saponin, flavonoid, steroid, terpenoid, glycoside, cardenolide, alkaloids, anthraquinone, and phenols.

Proximate analysis
Proximate analysis was assayed as described in Association of Official Analytical Chemists (AOAC) [9]. The leaf powder was analysed for crude protein, crude fat, crude fibre, ash, and moisture, and carbohydrate was calculated by difference.

Phytochemical quantification
Test for alkaloids
Alkaloids were measured as described in Soetan [10]. 0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides
Glycosides were determined as described by Sofowora [6]. To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for saponins
Saponins were determined using the method of Brunner [12]. To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigourously. And observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigourously after which it was observed for the formation of an emulsion.

Test for tannins
Tannins were measured using the method of AOAC [11]. About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration. Phlobatannins were assayed as described by Salau [14].

Test for phenols
Phenols were measured using the method of Mako [11].

Test for flavonoids
Flavinoids were measured using the method of Trease and Evans [7] Three methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappear on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicates the presence of flavonoids.

Test for terpenoids
Terpenoids were measured using the method of Trease and Evans (7). To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for anthraquinones
Anthraquinones were measured using the method of Trease and Evans [7]. 0.5 g of the extract was boiled with 10 ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Results
Alkaloids and saponins were present in appreciable amounts. Glycosides and phenols were present in moderate amounts while tannins, steroids, flavonoids, phlobatannins, and anthraquinones were present in minute quantities (Table 1). Cardenolides, terpenes were absent.

Table 1: Phytochemical screening of *Ocimum gratissimum* leaf

Parameters	
Alkaloids	+++
Saponins	+++
Tannins	+
Phlobatannins	+
Glycosides	++
Phenols	++
Anthraquinones	+
Cardenolides	−
Steroids	+
Terpenes	−
Flavonoids	+

+++appreciable amount; ++, moderate amount; +, a minute or trace amount; −, completely absent.

The proximate analysis indicated that the leaf powder had a high carbohydrate content 40.32% (Table 2). The crude protein and crude fibre content were 7.5% and 7.37%, respectively. The crude fat, ash, and moisture contents had the lowest percentages of 3.10%, 7.89%, and 8.00%, respectively (Table 2).

Table 2: Proximate and phytoquantitative analysis of *Ocimum gratissimum* leaf

Parameters	% Composition
A Proximate analysis	
Crude protein	7.5±0.20
Crude fat	3.10±0.04
Crude fibre	7.37±0.21
Ash	7.89±0.015
Moisture	8.00±0.032
Carbohydrate	40.32±0.05
B Phytoquantitative analysis	
Phytate	0.13±0.0014
Oxalate	0.15±0.0014
Tannin	0.23±0.0014
Saponin	0.52±0.0021
Alkaloid	0.29±0.0021
Phlobatannin	0.003±0.0014
Phenol	0.038±0.0021
Glycoside	0.03±0.0021

As observed in the phytochemical screening, quantitative analysis also indicated that alkaloids (0.29%) and saponins (0.52%) had the highest concentrations compared to the other phytochemicals that were quantified (Table 2). Phytates, oxalates, and glycosides were also present in moderate quantities with a concentration of 0.13%, 0.15%, and 0.03%, respectively. The phytochemical with the lowest concentrations was phlobatannin with a concentration of 0.003% (Table 2).

Discussion

Phytochemical screening of the plants revealed some differences in the constituents of the four plants tested by Ayoola *et al.* [9] C. papaya tested positive for all the phytochemicals tested; M. indica showed the absence of anthraquinones, alkaloids and cardiac glycosides; V. amygdalina tested positive for all except anthraquinones while P. guajava tested positive for all except Anthraquinones and alkaloids. All the plants exhibited potent antioxidant activity. The presence of flavonoids and tannins in all the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers [14]. Akinmoladun *et al.* [18] and Nweze and Eze [19] carried out the phytochemical screening of *O. gratissimum*; however, quantitative analysis of the individual phytochemical components were not studied. Also, their study did not screen for phenols, cardenolides, and chalcones. Akinmoladun *et al.* [18] also reported the absence of alkaloids in the aqueous extracts of the leaf, while this study confirmed the presence of alkaloids in the aqueous extracts of the leaf. Akinmoladun *et al.* [18] confirmed the presence of steroids, terpenoids, and flavonoids in the aqueous and ethanolic leaf extracts; however, these phytochemicals were found to be absent in this current investigation. Flavonoids were also reported to be absent in the ethanolic extract of the leaf [19]. Variation in the phytochemical content of the leaf extract could be due to planting location, seasonal variation, and extraction variables (temperature, time, concentration, and particle size). While screening 70 medicinal plant extracts for their antioxidant capacity and total phenols, Katalinic *et al.* have reported the presence of phenolic contents in different plants to the varying extents [23]. These compounds act as free radical scavengers and thus help protect cells from oxidative toxicity [24, 25]. Some workers have demonstrated the presence of phenolic compounds in the aerial parts of the plants including C. *procera, T. peruviana, and C. sativa* [26] but analysis of these phyto constituents in the aqueous extracts of different specific parts of these plants has not been worked out.

This study showed that the *O. gratissimum* leaf has a crude fat content of 3.10%. Essential oils that have been extracted from the oil of the leaf include eugenol, thymol, citral, geraniol, and linalool [20]. According to Lahlou [20], essential oils are poorly soluble in water, and the use of various solvents (such as acetone and ethanol) in the dilution of essential oils has been recommended.

Conclusion

The present study shows that the presence of flavonoids and tannins in all the plants is likely to be responsible for the free radical scavenging effects. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. The extraction of plant constituents is essential to isolate biologically active compounds and in understanding their role in disease prevention and treatment and in knowing their toxic effects as well.

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