Secondary Metabolites Constituents and Antibacterial Screening of Methanolic Leaves Extracts of *Senna fistula* and *Ocimum gratissimum*

[1]M.T. Ayinla, [2]S.B. Adeyemi, [1]B.V Owoyele and [3]R.Krishnamurthy

Departments of 1.Physiology, 2.Plant Biology, University of Ilorin, Nigeria.
3.C.G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Gujarat, India

Abstract

The misuse of antibiotics has contributed largely to the development of resistance strains thereby creating the need for an incessant search and development of newer drugs. This study investigated the secondary metabolites constituents and antibacterial activity of methanolic leaves extracts of *Senna fistula* (SF) and *Ocimum gratissimum* (OG). The SF and OG leaves extracts were done using methanol, the phytochemical analysis were done as per standard protocol. The antibacterial activity was determined by agar well diffusion method.

The results showed that the methanolic leaves extracts of SF and OG contain secondary metabolites like flavonoids, tannins, alkaloids, terpenoids, cardiac gylcosides, saponins, phenolic compounds. The antibacterial screening revealed that both *Senna fistula* and *Ocimum gratissimum* exhibited strong inhibitory activity against all the tested organisms. Methanolic leaf extract of SF has a stronger antibacterial activity with zones of inhibition of 19.5mm against *E coli* and 31.5 mm against *Klebsiella* than methanolic leaf extract of OG (18 mm against *E coli* and 28 mm against *Klebsiella*) in a dose dependent manner.

In conclusion, the methanolic leaves extracts of *Senna fistula* and *Ocimum gratissimum* exhibited antibacterial activity which is due to the presence of various secondary metabolites; therefore this study supports its use as an antibiotic agent.

Key words: *Senna fistula,* Antibacterial, Terpenoids, *Klebsiella, Ocimum gratissimum,* Secondary metabolites

Introduction

The availability of antimicrobial agents has brought a lot of benefit to mankind because it helps to curb large scale epidemics that could result in loss of lives.

Correspondence to:

M.T. Ayinla
Department of Physiology
Faculty of Basic Medical Sciences
College of Health Sciences
University of Ilorin, Ilorin, Nigeria
E-mail address: gazmark@unilorin.edu.ng
Mobile phone number: +234-8076029175

However, despite the existence of potent antimicrobial drugs, the misuse of antibiotics has contributed largely to the development of resistance strains thereby creating the need for an incessant search and development of newer drugs.

Ample source of potential antimicrobial are given to us as gift by nature in form of medicinal plants, and according to WHO, medicinal plants have been defined as any plant in which one or more of its organ contain substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs[1].

Senna fistula Linn. (*Senna*) family leguminousa. Popularly called Indian Laburnum, locally known as Aidantoro in Yoruba. It originated from India, but it is now grown in different countries of the world. It is a medium-sized tree growing up to 10-20 m tall. It has a deciduous leaves which is about 15-16 cm long and pinnate with 3-8 pairs of leaflets. Stem bark is pale grey, smooth and slender when young and dark brown, rough when old[2]. *Senna fistula* has been reported to be used in the treatment of cough[3], as laxative[4], its anti-diabetic[5], antioxidant and anti-inflammatory activities[6] have also been reported.

Ocimum gratissimum L. belongs to the family Lamiaceae. Is an aromatic perennial shrub that is native to Africa but now grown in different places in the world (India, Thailand, Vietnam). It is known as African Basil or Sweet basil in English and locally known as Efirin in Yoruba and Daidoya in Hausa languages. Its leaves are 2-4.5 cm long slender, pubescent, blade elliptical to ovate[2], flowers are branched with tolerably close whorls[7]. *Ocimum gratissimum* is used traditionally as stomachic, used in the treatment of headache and influenza. The seed has laxative property. Its essential oil is used against fever[2].

This study investigated the secondary metabolite constituents and antibacterial effects of methanolic leaves extracts of *Senna fistula* and *Ocimum graissimum* on some clinical isolates.

Materials and methods

The plant materials (leaves) of *Senna fistula* and *Ocimum gratissimum* were gotten from Abeokuta, Ogun state and Oro Town, Kwara state respectively. The identification of the plants was carried out in the Department of Plant Biology, University of Ilorin, Kwara state. Fresh leaves of *O. graissimum* and leaves

of *S. fistula* were air-dried at room temperature for about 2 weeks. The dried leaves of both *O. gratissimum* and *S. fistula* were pulverised using electric blender and kept in different plastic containers. Samples were transported to Uka Tarsadia University CGBIBT, Maliba campus, Gujarat, India.

Preparation of The extracts

About 200 g and 190 g of air-dried and powered leaves of *S. fistula* and *O. gratissimum* respectively were put in two separate 2000 ml conical flasks. 1000 ml of methanol was added into each of the 2000 ml conical flask, mixed and shaken thoroughly for about 30 minutes. The shaken was done daily for about five days. After five days, the mixtures were filtered using No. 4 whatman filter paper. The filterates were collected and evaporated to dryness using a well regulated water bath at 60^0C. A dark green extracts weighing about 21 g (10.5%) and 13 g (6.84%) were obtained for *Senna fistula* and *Ocimum gratissimum* leaves respectively. The extracts were stored in the refrigerator before the commencement of the study.

Test Organisms

Pure culture of experimental bacteria were obtained from Microbial Type Culture Collection (MTCC) Chandiarch, India. Test organisms used were *Salmonella, Bacillus megaterium, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonae.* Pure cultures of bacteria were maintained on nutrient agar medium. Each bacterial culture was maintained by subculturing regularly on same medium and stored at 4^0c before use in the experiment[8].

Method

Antibacterial Activities for the methanolic extracts of *Senna fistula* and *Ocimum gratissimum* were determined by agar well diffusion method. The pure bacterial cultures were prepared in nutrient broth for 24 hrs before use. 0.2 ml of the pure bacterial culture was spread on Mueller Hinton agar (MH) with sterile glass spreader[9]. Inoculum turbidity was maintained constant 0.8 OD at 660nm throughout the experiment. The level of turbidity is equivalent to approximately 1 X 108 CFU/ml[10]. Sterile 5 mm diameter of cork borer was used to bore well into the MH agar. 100µl (50 mg/ml and 100mg/ml) of each plant extract were introduced into the well separately and allowed to stand for 2 hrs and plate incubated at 37^0C. The plate were observed for zone of inhibition after 24 hrs and compared with standard drug[9] (Streptomycin).

Phytochemical screening of The methanolic leaves extracts of *Senna fistula* and *Ocimum gratissimum*

The Preliminary qualitative phytochemical screening for the presence of bioactive compounds such as flavonoids, terpenoids, cardiac glycosides, tannins, steroids, saponins, alkaloids, phlobathanins, phenolics and phytosterols were done using standard methods[11,12].

Results

Table 1 shows that methanolic leaf extract of *S. fistula* contains the following secondary metabolites: flavonoids, terpenoids, tannins, cardiac glycosides, phlobatannins and phenolics, while steroids, saponins and phytosteroids are absent. Also, the methanolic leaf extract of *O. gratissimum* contains flavonoids, tannins, steroids, saponins, phenolics and phytosteroids while, terpenoids, cardiac glycosides and phlobatannins are absent.

The methanolic leaves extracts of *S. fistula* and *O. gratissimum* showed inhibitory activities against all the tested organisms. The inhibitory activities of the two extracts occurred in a dose dependent manner with their zones of inhibition compared favourably with the standard drug (Streptomycin). The methanolic leaf extract of *S. fistula* showed stronger inhibitory activity on all the tested organisms (with the zones of inhibition ranging from 19.5 mm to 31.5 mm Figure 1) than the methanolic leaf extract of *O. gratissimum* (zones of

Table 1: Secondary Metabolites Constituents of Methanolic leaves extracts of *Senna fistula* and *Ocimum gratissimum*

Secondary metabolites	Methanolic leaf extract of *Senna fistula*	Methanolic leaf extract of *Ocimum gratissimum*
Flavonoids	+	+
Terpenoids	+	-
Cardiac glycosides	+	-
Tannins	+	+
Steroids	-	+
Saponins	-	+
Phlobatanins	+	-
Phenolics	+	+
Phytosterols	-	+

key: + = present; - = Absent

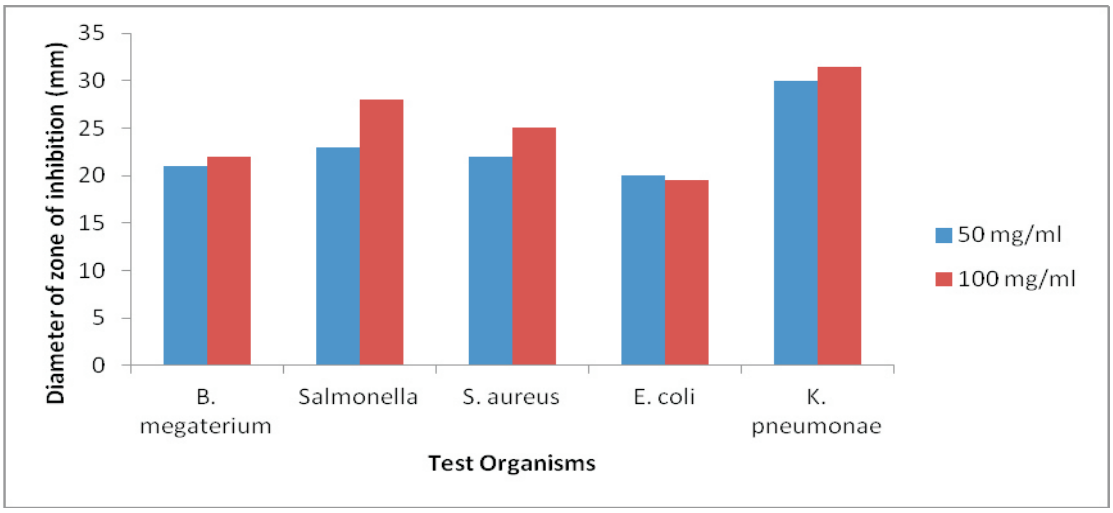

Figure 1: Zones of Inhibition on the Bacteria by the Methanolic leaf extract of *Senna fistula*

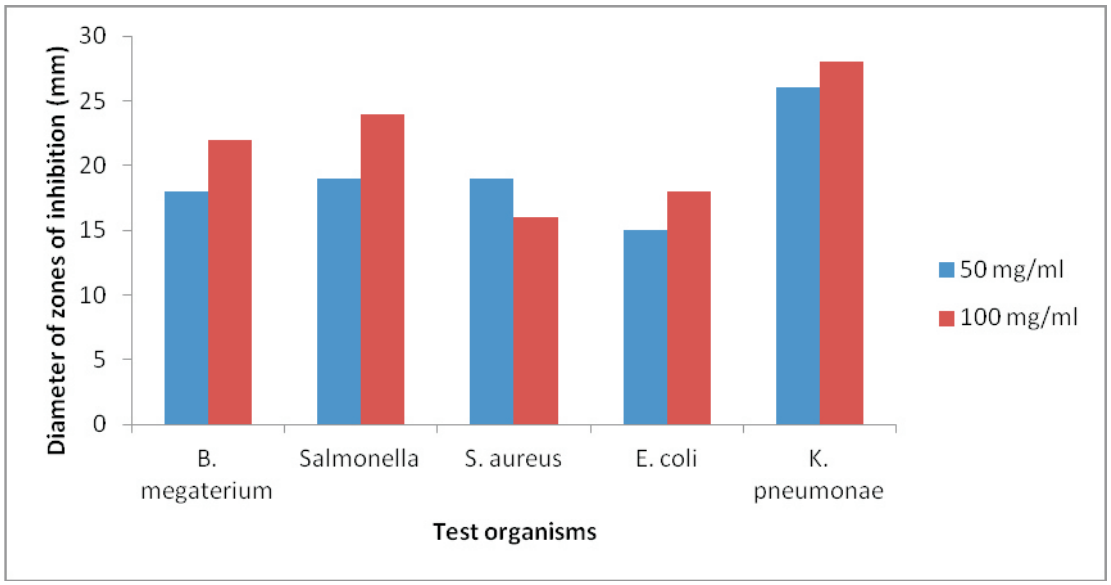

Figure 2: Zones of Inhibition on the Bacteria by the Methanolic leaf extract of *Ocimum gratissimum*

inhibition ranging from 15mm to 28 mm Figure 2). The Inhibitory activity of methanolic leaf extract of *S. fistula* is more pronounced against the two gram-negative bacteria (*E coli and K.pneumonae*) tested (Figure 1).

Discussion

Medicinal plants have been used in many cultures in the treatment of wide variety of diseases due to their easy accessibility and little or no side effects. The preliminary screening of the secondary metabolites of the methanolic leaves extracts of *S. fistula* and *O. gratissimum* revealed the presence of different constituents such as flavonoids, alkaloids, terpenoids, cardiac glycosides, tannins which have been reported to have curative effects on pathogens. Plant derived secondary metabolites are products of primary metabolism in plants, and are known to serve as a defence mechanisms against insects and many microorganisms[13]. In addition these secondary metabolites exhibit several biological activities such as anti-inflammatory, anticancer, antimicrobial[14, 15].

The choice of test organisms used in this study was based on the prevalence of the test organisms in causing infectious diseases, for example *S. aureus* is known to cause boils and *E coli* causes travellers' diarrhoea, also the number of test organisms used was based in literature[16, 17]. The zones of inhibition observed in the extracts treated colonies of the bacterial confer antibacterial effects of the two methanolic leaves extracts of *S. fistula* and *O. gratissimum*. This findings agree with previous studies documenting antimicrobial effects of ethanolic extracts of *S. fistula and O.* gratissimum in two separate studies[18, 19]. The sensitivity

of methanolic leaves extracts of *S. fistula* and *O. gratissimum* on all the investigated bacterial species and in dose dependent manner may be attributed to the presence of some secondary metabolites constituents that had inhibitory effects on microorganisms. In addition, the differences observed in the zones of inhibition of these leaves extracts suggest the susceptibility of the test organisms to different secondary metabolites present in the plants. Some of these compound (glycosides, alkaloid, flavonoids) have been reported to have antimicrobial activity using different mechanisms,[20] for example alkaloids act as antibacterial agent either by inhibiting nucleic acid synthesis, or by inhibiting cell division or by compromising outer membrane and cytoplasmic membrane integrity[21, 22]. Similarly flavonoids act as a bacterial agent by forming complex with proteins as well as destroying microbial membrane[23, 24]. Therefore, the presence of these phytoconstituents possibly contribute to the antibacterial effects of *S. fistula* and *O. gratissimum.* The antibacterial activity present in these plants may not necessarily due to a single component but a mixture of many secondary metabolites present in the extracts.

The emergence of resistance to antibiotics has led to increased morbidity and mortality, thus contributing to the world economic burden. WHO in its report stated that organisms like *E coli, Klebsella, Samonella* etc have a high rate of resistance against antibiotics[25, 26]. In this study methanolic leaf extract of *S. fistula* has a strong inhibitory activity against gram-negative bacteria (*E-coli, Klebsella),* therefore, the ability of this plant extract to inhibit the growth of these microorganisms demonstrates promising antibacterial activity of this plant, thus raising hope of eliminating antibiotic resistant microorganisms.

In conclusion, the two plant extracts have shown antibacterial activities which may be due to their secondary metabolites. Further studies should be carried out to isolate the compounds present in the plant to be able to determine the exact compound (s) that is contributing to the antibacterial activities of the plants.

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